STUDY OF DRINKING WATER QUALITY OF KATHMANDU METROPOLITAN AREAS AND EVALUATION OF ANTIBACTERIAL PROPERTY OF SOME MEDICINAL PLANTS AGAINST ISOLATED ENTERIC BACTERIA

A

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BY

ANUP MUNI BAJRACHARYA

CENTRAL DEPARTMENT OF MICROBIOLOGY

TRIBHUVAN UNIVERSITY

KIRTIPUR, KATHMANDU, NEPAL

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RECOMMENDATION

This is to certify that **Mr. Anup Muni Bajracharya** has completed this dissertation work entitled **"STUDY OF DRINKING WATER QUALITY OF KATHMANDU METROPOLITAN AREAS AND EVALUATION OF ANTIBACTERIAL PROPERTY OF SOME MEDICINAL PLANTS AGAINST ISOLATED ENTERIC BACTERIA "** as a partial fulfillment of Master of Science Degree in Microbiology under our supervision. To our knowledge, this work has not been submitted for any other degree.

Prof. Dr. Shital Raj Basnyat	**Dr. Kayo Devi Yami**	**Mr. Binod Lekhak**	**Ms. Tista Prasai**
Professor	Chief Science Faculty	Assistant Professor	Scientific Officer
CDM, TU	NAST, Khumaltar	CDM, TU	NAST, Khumaltar

Date:

CERTIFICATE OF APPROVAL

On the recommendation of **Prof. Dr. Shital Raj Basnyat, Dr. Kayo Devi Yami, Mr. Binod Lekhak** and **Ms. Tista Prasai,** this dissertation work of **Mr. Anup Muni Bajracharya** is approved for the examination and is submitted to the Tribhuvan University in the partial fulfillment of the requirement for Master of Science Degree in Microbiology.

_____

Dr. Anjana Singh, Ph.D.
Head of Department
Central Department of Microbiology
Tribhuvan University
Kathmandu, Nepal

Date:

BOARD OF EXAMINERS

Recommended by:

Prof. Dr. Shital Raj Basnyat
Supervisor

Mr Binod Lekhak
Supervisor

Dr. Kayo Devi Yami
Supervisor

Ms. Tista Prasai
Supervisor

Approved by:

Dr. Anjana Singh
Head of Department

Examined by:

External Examiner
Dr. Kanti Shrestha
Scientific Officer
Natural Product Research Laboratory
NAST

Internal Examiner
Dr. Shreekant Adhikari
Associate Professor
CDM, TU

Date:

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Anup Muni Bajracharya

Date:

Abstract

Present study was carried out to evaluate the quality of drinking water of the Kathmandu Metropolitan Areas in period of October 2005 to November 2006. A total of one hundred and fourteen water samples with thirty eight each from three different sources namely stone spouts, taps and tube wells were collected. The physico-chemical and microbiological analysis of the samples were conducted. The pH of 14.91 % of water samples were found to be below WHO guideline value. Similarly 24.56%, 26.32% and 31.58% of water samples were found to exceed WHO guideline value for conductivity, turbidity and iron content respectively. Hardness and the chloride content of all water samples were within the guideline value whereas 22.81% of samples crossed ammonia permissible level. The nitrate content of all samples except one tube well sample was found within permissible level. Similarly, seven water samples (6.14%) exceeded the WHO guideline for arsenic level in the study. The bacteriological analysis of water samples revealed the presence of total coliform in 90.35% of total samples (tube well-97.37 %, tap-73.68% and stone spout-100 %). Ten different kinds of enteric bacteria were isolated from the total contaminated samples. Among the isolates, *Citrobacter* spp (26.22%) was found to be maximum followed by *Escherichia coli* (25%), *Enterobacter* spp (20.73%), *Shigella* spp (8.54%), *Proteus vulgaris*(7.93%) *Pseudomonas aeruginosa* (3.66%), *Salmonella paratyphi* (3.05%) *Klebsiella* spp (2.44%), *Proteus mirabilis* (1.83%) and *Salmonella typhi* (0.61%). The water analysis data showed significant positive correlation between conductivity and hardness values for all water source types. Similarly, conductivity correlates significantly with chloride for tube well and stone spouts. The findings also showed a significant positive correlation of conductivity with nitrate, ammonia and iron in tap water and conductivity with nitrate in stone spouts. The turbidity and iron values for tap water exhibited significant positive correlation.

Eight different medicinal plants were screened and evaluated for their antimicrobial activity against the enteric bacteria isolated from water on the basis of their common use among the different ethnic groups for common disorder. Among them, *Punica granatum*, *Woodfordia fruticosa, Psidium guajava* and *Syzygium cumini* were found to be effective against all enteric bacteria whereas *Mimosa pudica, Acorus calamus, Aegle marmelos* and *Anethum sowa* were found to be ineffective against all.

The minimum bactericidal concentration (MBC) of these plant extracts found against *Salmonella typhi*, *Salmonella paratyphi*, *Proteus mirabilis* and *Proteus vulgaris* were lower (0.39-25mg/ml) so are more susceptible whereas the plants showed lethal effect against *Pseudomonas* spp, *Citrobacter* spp, *Enterobacter* spp, *E. coli*, *Shigella* spp. and *Klebsiella* spp. at MBC value of around 25-50mg/ml.

Key words: water**,** enteric bacteria, medicinal plants, minimum bactericidal concentration

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LIST OF ABBREVIATIONS

APHA : American Public Health Association
DISVI : Italian International Co-operation
DoHS : Department of Health Services
EDTA :Ethylenediamine tetraacetic acid
EHEC : Enterohemorrhagic *E. coli*
ENPHO : Environment and Public Health Organisation
EPA : Environmental Protection Agency
EWA : Environment Working Group
ETEC : Enterotoxigenic *E. coli*
HMG : His Majesty Government
ISRSC : Informal Sector Research and Study Centre
JICA : Japanese International Cooperation Agency
MBC : Minimum Bactericdal Concentration
MIC : Minimum Inhibitory Concentration
NAST : Nepal Academy of Science and Technology
NRA : National Research Associates
NTU : Nephelometric Turbidity Unit
NWSC : Nepal Water Supply Corporation
S.S : *Salmonella Shigella*
TCBS : Thiosulphate Citrate Bile Salt Sucrose
TSI : Triple Sugar Iron
UNICEF : United Nation International Children's Emergency Fund
US : United States
VDC : Village Development Committee
VP : Voges Proskauer
WHO : World Health Organization
WHO GV : World Health Organization Guideline Value
ZOI : Zone of Inhibition

CHAPTER-I

1 INTRODUCTION

Water is an exceptional gift of creation to the earth as it is the unique basis for life. It is essential for the well being of mankind and for sustainable development. The most precious resource of any nation is a healthy population. Improved health however, is impossible to achieve without adequate water supplies, safe drinking water and basic sanitation. So, clean, protected and safe water is absolute need for healthy productive life.

Though water is necessary for human survival, many are denied access to sufficient potable water supply and sufficient water to maintain basic hygiene. A large fraction of world's population around 1.1 billion do not have access to safe drinking water. The majority of these are in Asia and Sub-Saharan Africa. Furthermore, 2.6 billion people are living with no proper means of sanitation (WHO/UNICEF, 2000).

The drinking water supply in most of the rural areas and municipalities of Nepal are usually inadequate in terms of overall coverage, quantity of water and of course poor water quality. Kathmandu is the best example of this where people are struggling to get adequate water to meet the daily requirements and at the same time facing problem of unsafe water. Due to the inadequate and intermittent water supply, people in Kathmandu Valley rely on traditional water sources such as stone spouts, hand pumps and shallow wells to meet daily water need. In Kathmandu Valley, most of the sources of water can't be regarded safe and measured up to the guidelines recommended by WHO (Bottino, 1999; Prasai, 2002).

Water, although an absolute necessity for life can be a carrier of many water borne diseases such as typhoid, cholera, hepatitis, dysentery and other diarrhoeal related diseases. UNICEF estimates that over 80% of the world diseases are water borne. Children bear the greatest health burden associated with poor water and sanitation. Diarrhoeal diseases attributed to poor water supply, sanitation and hygiene account for 1.73 million deaths each year and contribute over 54 million disability adjusted life years, a total equivalent to 3.7% of the

global burden of disease (WHO, 2002). This place diarrhoeal disease due to unsafe water, sanitation and hygiene as the 6th highest burden of disease on a global scale, a health burden that is largely preventable (WHO, 2002).

Approximately 4 billion cases of diarrhoea each year cause 2.2 million deaths, mostly among children under the age of five which is equivalent to one child dying every 15 seconds. These deaths represent approximately 15% of all child deaths under the age of five in developing countries. Water, sanitation, and hygiene interventions reduce diarrhoeal disease on average by between one-quarter and one-third (Esrey, 1999; WHO, 2000).

The potential of drinking water to transport microbial pathogens to great numbers of people, causing subsequent illness, is well documented in countries at all levels of economic development. More recent outbreaks have involved *E. coli* O157:H7, the most serious of which occurred in Walkerton, Ontario Canada in the spring of 2000 and resulted in six deaths and over 2 300 cases (Bruce-Grey-Owen Sound Health Unit, 2000). The number of outbreaks that have been reported throughout the world demonstrates that transmission of pathogens by drinking water remains a significant cause of illness.

Outbreaks of water borne epidemic in Nepal is not uncommon. The incidence of such epidemics is considerably high in comparison with that of other diseases. Each and every summer water borne epidemics hit different parts of the country including Kathmandu City, the capital. Contaminated drinking water is one of the major routes for spreading such diseases. Annual report from DoHS (2004/2005) showed that there were 2,332 cases of typhoid, 18,611 cases of diarrhoeal diseases, 9,322 cases of intestinal worms, 543 cases of jaundice and infectious hepatitis in Kathmandu City.

The chemical quality of water is generally a far lower priority, as the majority of the impact of poor chemical quality is chronic rather than acute. However, there are some exceptions to this and some chemicals, notably iron, nitrate and recently arsenic is often included in routine monitoring of water supplies. Nitrate, fluoride and arsenic may also affect a large number of people depending on how they are released and the type of water supply (Speets, 2001).

Arsenic poisoning in the groundwater of Terai in Nepal is now becoming a new challenge for the nation's water supply sector. The few investigations that have been carried out on arsenic reveal that groundwater in Terai districts is contaminated with arsenic (ENPHO, 2003). Therefore, drinking water quality assessment has always been crucial with reference to public health importance in Kathmandu Valley.

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased (Nascimento *et al*, 2000). The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient.

For a long period of time, plants have been a valuable source of natural products for maintaining human health. According to WHO, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore such plants should be investigated to better understand their properties, safety and efficiency (Nascimento *et al*, 2000)

Medicinal plants have been used as traditional treatment for numerous human diseases for thousand of years. Water borne diseases continue to be a major cause of morbidity and mortality throughout the world. Thus, their treatment by using medicinal plant is an important public health issue. Medicinal properties of plants are due to the active chemical constituents present in different parts of the plant (Mitscher *et al*, 1980). Generally these active components are extractable with different kinds of solvents. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils as well as tannins and

other constituents include alkaloids, flavonoids, steroids, resins, fatty acids etc. The drinking water problem is a current and happening problem in Kathmandu, getting intensified with the rapid rate of urbanization and industrialization. Water borne epidemics are regular phenomenon due to poor water and sanitation facilities where the poor and marginalized people are most affected. The lack of studies in these sectors may have serious consequences in terms of morbidity and mortality. This study is thus conducted to reveal the status of the drinking water sources and is believed to be helpful in taking necessary steps for its improvement. Plant extracts have great potential as antimicrobial compounds against microorganisms. In remote rural areas of Nepal, many tribal societies utilize indigenous plants as an exclusive means of combating humans as well as animal diseases (Prasai, 2002). But despite of all these virtues, only few scientific studies have been conducted regarding indigenous plants for investigation to cure the certain infectious diseases. Consequently, this study was carried out so that the outcome would be useful for researchers, health planners and concerned people.

CHAPTER-II

2 OBJECTIVES OF THE STUDY

2.1 General Objective

To assess the drinking water quality of Kathmandu metropolitan areas and antimicrobial evaluation of some medicinal plants for their anti-enteric potential against enteric bacteria isolated from the water sample.

2.2 Specific objectives

- To study physico-chemical parameters of drinking water from different sources.
- To analyze bacteriological parameters of drinking water from different sources.
- To screen and evaluate antibacterial activity of crude ethanol extracts of some medicinal plants.
- To find out the minimum bactericidal concentration (MBC) value of crude ethanol extracts against isolated enteric bacteria from water.

CHAPTER-III

3 LITERATURE REVIEW

Nepal though rich in water resources, still suffers from water related problems. National water supply coverage is said to have increased substantially in the past decade yet people are still spending hours to get a bucket of water in both rural and urban communities including the capital city. Increasing water demand, shortage of clean drinking water and pollution of water resources are common phenomena of urban development (ENPHO, 2003).

Although almost 90% Kathmanduties have access to municipality piped water supply, people are still suffering from diarrhoeal diseases. Several researches have already pointed out that piped water supply in Kathmandu City is unsafe for drinking. Diarrhoea and gastroenteritis are the major problems in developing countries like Nepal due to unsafe and inadequate water supplies and sanitation, little or no health education, illiteracy, under nutrition, wide spread faecal contamination of environment, dense population etc (Chand *et al*, 2001). In Nepal, the reported cases of water borne communicable diseases were typhoid (2,15,191), diarrhoeal diseases (9,21,901), intestinal worms (6,11,072) and jaundice and infectious hepatitis (25,686) in 2004/2005 (DoHS, 2004/2005).

3.1 Physico-chemical parameters of water

The ordinary consumer judges the water quality by its physical characteristics. The provision of drinking water that is not only safe but also pleasing in appearance, taste and odour is a matter of high priority (Park, 2005).

3.1.1 Temperature

The temperature is basically important for its effects on the chemistry and biological reactions in the organisms in water. A rise in temperature of the water leads to reduce solubility of gases and amplify tastes and odours (Trivedy and Goel, 1986). High water temperature enhances the growth of microorganisms and may increase taste, odour, colour, and corrosion problem. Water in the temperature range of 7°C to 11°C has pleasant taste and

refreshing. Thus, cool water is generally more palatable than warm water (WHO, 1993). Temperature of water bodies undergoes variation along with normal climatic fluctuations. These variations occur seasonally and, in some water bodies, over periods of 24 hours. Temperature of surface water is influenced by latitude, altitude, season, time of day, air circulation, cloud cover and the flow and depth of water body.

3.1.2 pH

pH measurement is one of the most important and frequently used tests in water chemistry (APHA, 1998). pH less than 7.0 may cause corrosion and encrustation in the distribution system while disinfection with chlorine is less effective if pH exceeds 8.0. Uncontrolled pH of the water can result in the contamination of drinking water and in adverse effects in its taste, odour and appearance (WHO, 1993). The pH value of drinking water from any sources should be within range 6.5 - 8.5 (Trivedy and Goel, 1986).

3.1.3 Electrical conductivity

Conductivity in water is affected by the presence of inorganic dissolved solids such as chloride, nitrate, sulfate, and phosphate anions (ions that carry a negative charge) or sodium, magnesium, calcium, iron, and aluminum cations (ions that carry a positive charge). Organic compounds like oil, phenol, alcohol, and sugar do not conduct electrical current very well and therefore have a low conductivity when in water (APHA, 1998). The conductivity of distilled water ranges between 1 to 5 µS/cm but the presence of salts and contamination with wastewater increases the conductivity of the water. Consequently, a sudden rise in conductivity in the water will indicate addition of some pollutants to it (Trivedy and Goel, 1986).

3.1.4 Turbidity

Turbidity is a measure of the degree to which the water looses it’s transparency due to the presence of suspended particulates. It is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted with no change in direction or flux level through the sample (APHA, 1998). Turbidity in drinking water is caused by particulate matter (WHO, 1993). High levels of turbidity can protect microorganisms from the effects of

disinfections and can stimulate bacterial growth. WHO establishes that the turbidity of drinking water shouldn't be more than 5 NTU, and should ideally be below 1 NTU.

3.1.5 Hardness

Originally hardness of water was understood to be a measure of the capacity of water to precipitate soap. Soap is chiefly precipitated by the calcium and magnesium ions present. Hardness in water is caused by dissolved calcium and to a lesser extent, magnesium. It is usually expressed as the equivalent quantity of calcium carbonate (WHO, 2004). Depending on pH and alkalinity, hardness above about 200 mg/litre can result in scale deposition, particularly on heating. No health-based guideline value is proposed for hardness. However, the degree of hardness in water may affect its acceptability to the consumer in terms of taste and scale deposition.

3.1.6 Chloride

It is a major mineral constituent, generally found in the form of sodium (NaCl), potassium (KCl) and calcium ($CaCl_2$) salts. High concentration of chloride gives an undesirable taste to water and beverages. High chloride concentrations are corrosive to metals in the distribution system, particularly in waters of low alkalinity. Increase of chloride indicates the industrial domestic wastewater pollution.

3.1.7 Ammonia

Sewage contains large amount of ammonia formed by bacterial decay of nitrogenous organic wastes. Hence, surface water showing a sudden increase in the ammonia content may indicate sewage pollution or industrial pollution from dairies, abattoirs, tanneries or chemical plants. Ground water often contains some ammonia due to natural processes from reduction of nitrates by bacteria etc, but sudden changes in ammonia content may be due to the seepage of waste water. Ammonia will increase the chlorine demand of raw water in the chlorination process of disinfection. Its presence is not proof of contamination but may provide the supporting evidence of pollution.

3.1.8 Nitrate

The presence of nitrate and nitrite in water may result from the excessive application of fertilizers or from leaching of wastewater or other organic wastes into surface water and groundwater. It is an end product of the decay of nitrogenous material such as nitrate fertilizers or animal and human excreta. Nitrate in the ground water may be due the following causes.

1. Wastewater from households and industries
2. Spread of manure and fertilizers on land
3. Bacterial conversion of ammonium via nitrite into nitrate by oxidation

High nitrate content affects the blood's ability to counteract oxygen. Consumption of water containing high nitrate may cause death of infants by cyanosis (methaemoglobinaemia). Also, exposure to high nitrate content increases the risk of stomach cancer.

3.1.9 Iron

Iron is the most abundant metal in the earth's crust. It is found in natural fresh water at levels ranging from 0.5 to 50 mg per litre. In drinking water supplies iron (II) salts are unstable and are precipated as insoluble iron (III) hydroxide, which settles out as a rust-coloured silt. Anaerobic ground water may contain iron (II) at concentrations of up to several milligrams per litre without discolouration or turbidity in the water when pumped directly from a well, although turbidity and colour may develop in piped systems at iron levels above 0.05-0.1 mg/litre. Staining of laundry and plumbing may occur at concentrations above 0.3 mg/litre. Iron also promotes undesirable bacterial growth ("Iron bacteria") in water works and distribution system, resulting in the development of a slimy coating on the pipes (WHO, 1993).

3.1.10 Arsenic

Arsenic is a metalloid element present naturally in the earth's crust and forms chemical and organic complexes together with other metals, carbon and oxygen. Dominant natural arsenic bearing rocks includes realgar (AsS), orpiment (As_2S_3), arsenopyrite (FeAsS) etc. Due to several geo-physical events and natural chemical reactions, especially the oxidation and reduction processes, several arsenic compounds in soluble forms get released inside the earth

crust and contaminate the ground water. Anthropogenic sources of arsenic are numerous. They include the application of arsenical pesticides on land, incineration of arsenic containing substances, industrial discharges and manufacturing and use of wood preservatives and pesticides and paint industries may elevate concentration of arsenic in water and soil.

Arsenic has been classified as a group A carcinogen, also known as human carcinogen by the US Environmental Protection Agency (EPA 1998).The toxicity of arsenic depends upon its chemical form; arsenite (As^{3+}) is more toxic than arsenate (As^{5+}). Chronic arsenic poisoning may result from the accumulation of arsenic compounds in the body through arsenic contaminated drinking water and foods. Severe poisoning can arise from the ingestion of as little as 100 mg arsenic trioxide; chronic effect may results from the accumulation of arsenic compounds in the body at low intake levels (APHA, 1998).

3.2 Microbiological parameters of water

Water intended for drinking must be free from agents of waterborne disease. However, it's impracticable to test every pathogen, including bacteria, viruses and parasites that might be present in drinking water, since the methods are often difficult, expensive and time consuming. For this reason, in routine testing, microbial indicators of water quality i.e the normal intestinal organisms as indicators of faecal pollution are used, since their presence shows that pathogens could also be present. Coliforms organisms (total coliforms and faecal coliforms) are often used as indicator organisms of faecal pollution for monitoring and assessing the microbial quality of public water supplies.

3.2.1 Coliform bacteria

The term "coliform organisms" refers to Gram negative, oxidase negative, non-sporing rods capable of growing aerobically on agar medium containing bile salts and able to ferment lactose within 48 hours at 35-37^{0}C with the production of both acid and gas (Cheesbrough, 1993).

Coliform organisms have long been recognized as a suitable microbial indicator of drinking water quality, largely because they are easy to detect and enumerate in water. The levels of coliform organisms present in the drinking water should not exceed the maximum permissible value of less than one cell per 100 ml of water set by the World Health Organization. Coliform bacteria belong to the genera *Escherichia, Citrobacter, Enterobacter* and *Klebsiella* (WHO, 1993).

3.2.2 Some intestinal pathogens found in contaminated drinking water.

A. *Escherichia coli* pathogenic strains

Escherichia coli is present in large numbers in the normal intestinal flora of humans and animals, where it generally causes no harm. However, in other parts of the body, *E. coli* can cause serious disease, such as urinary tract infections, bacteraemia and meningitis. A limited number of enteropathogenic strains can cause acute diarrhoea. Several classes of enteropathogenic *E. coli* have been identified on the basis of different virulence factors, including enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC). EHEC serotypes, such as *E. coli* O157:H7 and *E. coli* O111, cause diarrhoea that ranges from mild and non-bloody to highly bloody, which is indistinguishable from haemorrhagic colitis. (WHO, 2004)

B. *Klebsiella* spp.

Klebsiella spp. are Gram-negative, non-motile bacilli that belong to the family Enterobacteriaceae. The outermost layer of *Klebsiella* spp. consists of a large polysaccharide capsule that distinguishes the organisms from other members of the family. *Klebsiella* spp. are excreted in the faeces of many healthy humans and animals, and they are readily detected in sewage-polluted water. (WHO, 2004)

Klebsiella spp causes chest infections and occasionally severe bronchopneumonia with lung abscesses. Infections are often opportunistic, occurring in those with existing chest disease or diabetes mellitus, or in malnourished persons Approximately 60–80% of all *Klebsiella* spp.isolated from faeces and clinical specimens are *K. pneumoniae* and are positive in the thermotolerant coliform test. *Klebsiella* can cause nosocomial infections, and contaminated

water and aerosols may be a potential source of the organisms in hospital environments and other health care facilities. (Collee *et al*, 1996)

C. *Citrobacter* spp.

It belongs to the family Enterobacteriaceae. They are gram-negative, motile rods (by peritrichous flagella, facultative anaerobic and optimal temperature required for growth is 37^0C. *Citrobacter species* may be isolated from human or animal faeces, from various clinical specimens and from food, water, sewage, soil etc (Singleton *et al.*, 2001).*Citrobacter* may be opportunist pathogens. *Citrobacter freundii* and *Citrobacter diversus* have been associated with cases of human diarrhoea. (WHO, 2004)

D. *Enterobacter* spp.

It belongs to the family Enterobacteriaceae. They are gram-negative motile, non-capsulated rods, lactose fermenting, aerobic and facultative anaerobic and grow at optimum temperature 37^0C. *Enterobacter* can be found in the intestinal tract of humans and animals and in soil, sewage, water and dairy products.*Enterobacter* are opportunistic pathogens associated with urinary infections, wound infections and septicemia especially in persons already in poor health.

E. *Salmonella* spp.

Salmonella spp. belongs to the family Enterobacteriaceae. They are motile, Gram negative bacilli that do not ferment lactose, but most produce hydrogen sulfide or gas from carbohydrate fermentation. *Salmonella* are excreted in the faecas of infected humans or animals. *Salmonella* infections typically cause four clinical manifestations: gastroenteritis (ranging from mild to fulminant diarrhoea, nausea and vomiting), bacteraemia or septicaemia (high spiking fever with positive blood cultures), typhoid fever / enteric fever (sustained fever with or without diarrhoea) and a carrier state in persons with previous infections. In regard to enteric illness, *Salmonella* spp. can be divided into two fairly distinct groups: the typhoidal species/serovars (*Salmonella typhi* and *Salmonella paratyphi*) and the remaining non-typhoidal species/serovars. (WHO, 2004)

F. *Shigella* spp.

Shigella spp. are Gram-negative, non-spore-forming, non-motile, rod-like members of the family Enterobacteriaceae, which grow in the presence or absence of oxygen. There are four species: *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*. *Shigella* spp. can cause serious intestinal diseases, including bacillary dysentery. All species can produce severe disease, but illness due to *S. sonnei* is usually relatively mild and self-limiting. In the case of *S. dysenteriae*, clinical manifestations may proceed to an ulceration process, with bloody diarrhoea and high concentrations of neutrofils in the stool. (WHO, 2004)

G. *Proteus* spp.

Proteus spp. are Gram-negative, actively motile, non-capsulated lactose non fermenting members of the family Enterobacteriaceae which hydrolyse urea rapidly.(WHO, 2004) *Proteus spp* are found widely distributed in soil, polluted water, intestine of healthy man and animals. *Proteus mirabilis* is the commonest species of *Proteus* in human infection which cause urinary tract infection. It is often found in domiciliary patients with diabetes or structural abnormalities of the urinary tract and hospital patients after instrumentation. *Proteus vulgaris* may cause urinary infection. (Collee *et al*, 1996)

H. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a member of the family Pseudomonadaceae and is a polarly flagellated, aerobic, Gram-negative rod. When grown in suitable media, it produces the non-fluorescent bluish pigment pyocyanin. Many strains also produce the fluorescent green pigment pyoverdin. (WHO, 2004) *Pseudomonas aeruginosa* is a common environmental organism and can be found in faeces, soil, water and sewage. It is a classic opportunistic pathogen with innate resistance to many antibiotics and disinfectants and causes skin infection especially at burn sites, wounds, respiratory infection, otitis external, eye infection usually hospital acquired and septicemia (Cheesbrough, 2000).

I. *Vibrio* spp.

Vibrio spp. are small, curved (comma-shaped), Gram-negative bacteria with a single polar flagellum. *Vibrio cholerae* is the only pathogenic species of significance from freshwater

environments. While a number of serotypes can cause diarrhoea, only O1 and O139 currently cause the classical cholera symptoms in which a proportion of cases suffer fulminating and severe watery diarrhoea. Strains of *V. cholerae* O1 and O139 that cause cholera produce an enterotoxin (cholera toxin) that alters the ionic fluxes across the intestinal mucosa, resulting in substantial loss of water and electrolytes in liquid stools.

Cholera outbreaks continue to occur in many areas of the developing world. Symptoms are caused by heat-labile cholera enterotoxin carried by toxigenic strains of *V. cholerae* O1/O139. Non-toxigenic strains of *V. cholerae* can cause self-limiting gastroenteritis, wound infections and bacteraemia. (WHO, 2004)

3.3 Global scenario of drinking water quality

Access to water supply and sanitation is a fundamental need and a human right. It is vital for the dignity and health of all people. Globally, 1.1 billion people are without access to safe water supply and 2.4 billion are without access to improved sanitation. Figures 1 and 2 show the global population deprived of proper sanitation and improved drinking water supply. Majority of the people undergoing the situation live in Asia (WHO/UNICEF, 2000).

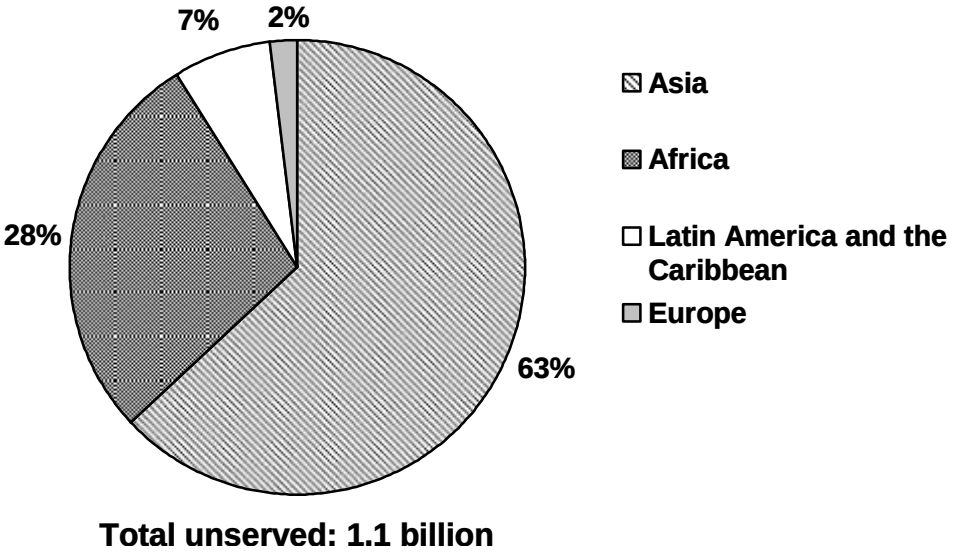


Figure 1 Distribution of the global population not served with safe water supply, by region (WHO/UNICEF, 2000)

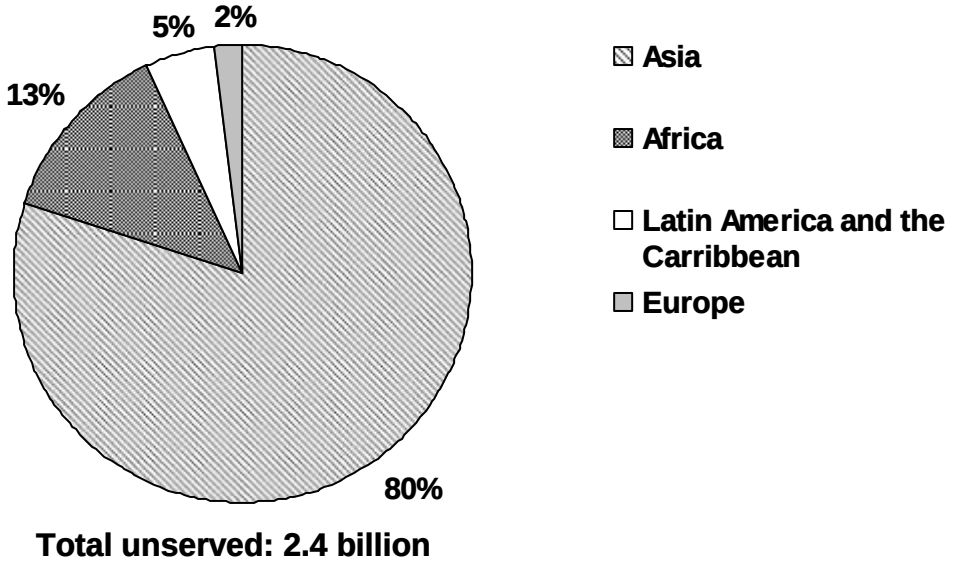


Figure 2 Distribution of the global population not served with improved sanitation, by region (WHO/UNICEF, 2000)

A study conducted by Favinka (1981) in rural communities of Mutenda found that the bore hole water was the only source of supply bacteriologically safe for drinking. The need for further research on the microbiology of surface and underground water resources was stressed by the study. Mascher and Reinthaler (1987) reported that the populations of Ogun State, Nigeria were not provided with drinking-water of suitable quality, as defined by international standards. It was attributed to the secondary contamination, due to inappropriate transport and storage of the water which led to further reduction in the quality of the already contaminated water.

Lindskog and Lindskog (1988) examined the bacteriological quality of drinking water sources and of stored household water in a rural area of Malawi, before and after improvement of the method of water supply. Among the traditional water sources, water quality was better in springs than in wells and rivers. During the rainy season, there was a considerable deterioration of water quality, which were most pronounced in wells. The improved water supply system consisted of piped, untreated surface water from an uninhabited mountain area. This water contained a mean value of 54 faecal coliforms per 100 ml which can be regarded as acceptable in this setting.

An investigation carried out on drinking water quality in rural areas of 180 counties in 26 provinces, municipalities and autonomous regions of China revealed that 42.7% of populations were supplied with unqualified drinking water. The bacteriological indices of drinking water exceeded the standard seriously. Organic pollution occurred extensively. Some regions supplied with water of high concentration of fluoride. (Zhang, 1997)
Nola *et al* (1998) in the Yaounde, Cameroon carried out a microbiological survey of five spring water points and ten wells and found that these water supplies contained many types of bacteria including *Pseudomonas aeruginosa*, *Aeromonas hydrophila* and indicators of fecal contamination.

A bacteriological analysis of drinking water conducted in the Shimla (India). *Salmonella typhi* was isolated in 1.25% samples by membrane filtration technique. The water from all the natural sources was unfit for human consumption whereas piped water supply was of good quality in general (Grover and Thakur, 2001).

The presence of excess arsenic in ground water has been reported from West Bengal (India). Nearly, 13.8 million people in 75 blocks are reported at risk. It is also reported that around 0.2 million people in West Bengal have arsenic related skin manifestations. Other water quality problems in India include varying iron levels in groundwater (which restricts water utility owing to colour, turbidity and taste), especially in northeastern India. Similarly nitrates and other heavy metal contamination and bacteriological contamination have been observed (India assessment, 2002).

A study of bore well water of Mysore city (India) has revealed that 47.66% contain nitrate greater than 45 ppm. *Klebsiella ozaene*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella* spp. and *Citrobacter freundi* were isolated as the faecal contaminants in the study by Nagaraju and Sastri (2004). A study by Anwar *et al* (2004) in Lahore (India) revealed overall bacterial contamination rate of 20.64% and among chlorinated samples, 12.32% showed bacterial contamination. Venus (2005) has found 33% faecal contamination rate in Vocin. All of the stored drinking water samples were found coliform positive in two

villages in the rural district of Chikwawa in Southern Malawi. *E. coli* was isolated from 91% and 80% of water samples in two villages (Jabu, 2005).

In an analysis of more than 22 million tap water quality tests, most of which were required under the federal Safe Drinking Water Act, EWG found that water suppliers across the U.S. detected 260 contaminants in water served to the public. One hundred forty-one (141) of these detected chemicals more than half are unregulated; public health officials have not set safety standards for these chemicals, even though millions drink them everyday (Environmental Working Group, 2005).

Rajendra *et al* (2006) has reported the presence of coliforms in 37% water samples in a study conducted in the tsunami affected villages and relief shelters of Tamilnadu. One isolate each of *Salmonella paratyphi* B and NAG *Vibrio* were isolated from two well water samples in the same study.

3.4 National scenario of drinking water quality

3.4.1 Studies on physico-chemical parameters

ENPHO/DISVI (1990) examined water quality in Terai Tubewell Project in seven rural areas of the Eastern Development Region of Nepal. Most of the parameters (appearance, odour, pH, conductivity, calcium, magnesium, chloride, ammonia and oxygen consumed) were found within the WHO guidelines, while iron and hardness exceeded the value. ENPHO/DISVI (1991) analysed water quality in Siraha district. Average values for pH, total hardness and chloride were found within WHO acceptable limit. Average conductivity ranged from 461-1,313 µS/cm, and ammonia from 0.2-1.1 mg/l and iron from 0.5-7.1 mg/l.

In a study conducted by Bottino *et al* (1991) in Kathmandu revealed that the most water samples were within the permissible value regarding the chemical parameters whereas ammonia and nitrate concentration were higher than the limit in some samples. The iron concentration in most of the samples of the tap waters, reservoirs and treatment plants were higher than the permissible values.

Pradhananga *et al* (1993) examined water samples of stone spouts of Pashupati area in 1990 and 1992. In 1990 the average pH, ammonia and iron values ranged from 6.3-6.5, <0.05-0.56 mg/l and 0.05-0.84 mg/l respectively. The average values for conductivity, hardness and chloride lie within the WHO permissible level. Likewise, in 1992, the temperature and pH ranges from 21.4^{0}C - 23.4^{0}C and 6.3-6.7 respectively. Parameters like conductivity, hardness, chloride and turbidity were recorded within permissible level. Average values for iron and ammonia ranged from 0.2-1.9 mg/l and <0.01-3.02 mg/l respectively.

Ghimire (1996) assessed 11 groundwater samples from Patan area in two seasons. In rainy season, the pH and temperature ranged from 5.6-6.3 and 19.7-22.5^{0}C respectively. Conductivity, hardness and ammonia, for all samples were found to lie within WHO permissible level. Similarly, in summer season, pH and temperature ranged from 5.9-6.7 and 21^{0}C -22.7^{0}C. The values for conductivity and hardness were found within the WHO limit, whereas chloride, ammonia, total iron, calcium and magnesium ranged from 50-324 mg/l, 0.13->1.5 mg/l, <0.05-5.15 mg/l, 33.6-83.2 mg/l and 4.38-35.57 mg/l respectively.

Thapa (1997) recorded most of the parameters analysed within WHO standard for drinking water except BOD value of some drinking water samples. Temperature, pH, total hardness, chloride were found within safe limits set by WHO, 1993.

Maharjan (1998) recorded that most of water samples showed values within the WHO standard limit for drinking water. The values for turbidity, conductivity, hardness, iron, ammonia and chloride were found above maximum permissible levels for 12.9 %, 5.7 %, 1.4%, 12.9 %, 38.6 % and 1.4 % of samples, respectively.

Bottino *et al* (1999) carried out the study regarding the water quality of the stone spouts and reported that chemically water from the stone spouts can be consumed for drinking, however there are few exceptions like Bandganga and Chabahil stone spout in case of pH and Tamchhinpakha stone spout in case of iron.

Prasai (2002) assessed 132 samples from Kathmandu City and reported that 8.3% of water samples have crossed the guideline value for pH. Similarly, for conductivity, turbidity and iron content 43.2%. 81.1% and 41.7% of water samples have crossed the permissible guideline value and arsenic was not detected from any of the samples.

Joshi and Baral (2004) assessed a total of 163 water samples, randomly collected from from 86 tube wells and 77 open wells from urban areas of Kathmandu valley. The temperature of tested groundwater sources ranged from 14.7^0C to 27.4^0C and average pH records were found between 6.5 to 7.5. Turbidity measurements of 21% samples exceeded WHO guideline value of 5 NTU. Hardness values of 9% samples exceeded maximum permissible limit set by Nepal. However, electric conductivity of 79% of tested samples crossed United States Public Health (USPH) standard. Total iron concentration level of ground water of the valley was merely high and that of 48.5% of samples crossed WHO guideline. However arsenic contamination was not detected in any of the tested groundwater samples.

A study conducted jointly by JICA/ENPHO (2005) showed that out of total 134 samples tested in pre-monsoon, the pH value for one-third of the water samples (34.3%) was found to be below WHO GV, 65.7% samples within WHO GV and none of the sample exceeded the maximum WHO permissible limit. Majority of water samples exceeded the WHO GV of 0.3mg/L for iron (97.0%), 1.5mg/L for ammonia (85.8%) and 0.1mg/L for manganese (84.3%). The chloride and nitrate content, for all the water samples, was found within the permissible level for drinking water.

Ground water quality study in Kathmandu and Lalitpur municipality areas undertaken by JICA/ENPHO (2005) revealed that majority of the water samples exceeded the WHO guideline value of 0.3mg/l for iron, and 1.5 mg/l for ammonia in all the water sources types. The chloride content for all the water samples was found within the permissible level of 250 mg/l. In phase I, 50% of deep wells, 6.3% of shallow tube wells and 14.3% of shallow dug wells were found with arsenic concentration above WHO guideline value of 0.01 mg/l, while it was 68.6%, 11.4% and 11.5% in phase II, respectively.

3.4.2 Studies on microbial quality

ENPHO/DISVI (1990) conducted a study on water quality of 21 stone spouts of the Kathmandu city. Bacteriologically, samples from all the spouts have shown faecal contamination. The faecal coliforms densities were observed in the range of 1 to 37,602 col/100ml of water. Out of total, 81 % of the stone spouts showed very high contamination (>100 col/100ml) and 19% exhibited less than 100 col/100ml.

A study done by Karmacharya *et al* (1991/1992)in the Kathmandu showed that out of 172 samples from 5 treatment plants and public taps, 50% of water samples of city water supply were found contaminated with coliform.

ENPHO/DISVI (1992) conducted a one year monitoring on microbiological quality of water supply in the Kathmandu City. 39 samples from 5 treatment plants and 172 samples from 37 public taps were examined from different localities. Seven samples that is, 18% from treatment plants were found contaminated with an average faecal coliform of 4 col/100ml. Similarly, 50% samples from public taps were found contaminated. The bacterial densities in contaminated samples ranged from 1 to TMTC col/100ml.

Bottino *et al* (1991) recorded that the reservoirs and treatment plants having nil coliform were greater than 70 % of the measured samples except in the case of Sundarighat and Balaju treatment plants. In case of tap waters, total coliform counts in most of the samples are high, which indicates the contamination in the distribution system.

Pradghananga *et al* (1993) examined water samples from 6 different stone spouts around Pashupati area on three occasions during 1992. Average values of coliform at *Ban Binayak* (44 col/100ml), *Arun Dhara* (88 col/100ml), *Barun Dhara* (43 col/100ml), *Ganga Hiti* (10 col/100ml), *Mitra Dhara* (8 col/100ml), and *Ba Hiti* (61 col/100ml) were recorded.

Sharma (1993) carried out bacteriological examination of the potable water of different urban and rural areas of Nepal. The results showed the maximum densities of coliforms and faecal coliforms as 48×10^2 col/100ml and 240 col/100ml respectively in Kathmandu, 240 col/100ml

and 93/100ml in Hetauda, 48×10^2 col/100ml in Birgunj, Pokhara and Biratnagar. Some of enterobacteria were also isolated from water samples. The study indicated that in rural area 33.3 to 16.7% of water samples and in urban area 70 to 100% samples were found to be contaminated with coliform bacteria.

Ghimire (1996) studied the water quality of 6 stone spouts and 5 dug wells at the Patan area during two seasons. The study reported 2 stone spouts uncontaminated, 1 spout showed 1000 col/100ml, 2 spouts with more than 1,000 col/100ml, and 1 spout was dry during summer. One dug well was free of contamination and rest 4 wells were highly contaminated (100-7,000 col/100ml). All the spouts and wells were heavily contaminated during rainy season (4-2,600 col/100ml).

Thapa (1997) examined water quality of nine different sites in Baluwa VDC, near to the Kathmandu City. Water samples were collected during three seasons and from different sources. All the samples were found contaminated during all the seasons. The bacterial densities ranged from 43-210 col/100ml during winter, 75-240 col/100ml during summer and 150-460 col/100ml during rainy season.

Maharjan (1998) examined 70 water samples randomly collected from 5 shallow pumps, 3 shallow wells, 14 stone spout and 48 dug wells in urban area of Patan city were analysed. Out of these, 85.6% of samples showed presence of total coliforms and 68.6% contained faecal coliforms. In this study, 120 enteric bacteria were isolated from 49 samples. Recovery of *Enterobacter* spp. was maximum followed by *E. coli* > *Citrobacter* spp. > *Salmonella* spp. and others.

Bottino *et al* (1999) carried out the study regarding the water quality of the stone spouts in Kathmandu city and reported that bacteriologically all the samples shown faecal contamination in both seasons, hence water from all the stone taps were unfit for drinking.

Prasai (2002) assessed 132 water samples from Kathmandu City and reported that total bacterial count and coliform count showed that 82.6% and 92.4% of samples have crossed

WHO guideline value. In the study, 238 enteric bacteria were isolated from different sources. Percentage recovery of *E. coli* (26.4%) was found to be maximum followed by *Enterobacter* spp. (25.6%), *Citrobacter* spp. (22.6%), *Pseudomonas aeruginosa (*6.3%), *Klebsiella* spp. (5.4%), *Shigella* spp. (3.78%), *Salmonella typhi* (3.3%), *Proteus vulgaris* (2.9%), *Serratia* spp. (2.52%) and *Vibrio cholerae* (0.84%).

Joshi and Baral (2004) carried out the study regarding Chemical and Microbial quality of ground water of Kathmandu valley and reported that more than 87% of analysed groundwater samples of tube wells and open wells were found to be contaminated with faecal pollution indicator organisms "coliform bacteria" and hence were unfit for drinking.

A study conducted jointly by JICA/ENPHO (2005) in Kathmandu valley showed that out of total 134 tube wells water samples tested, 20.1% were contaminated with *E coli*, which ranged from 0-640 cfu/100ml of sample despite of the increased depth of wells.

3.5 Medicinal Plants

In developing countries in particular, a large percentage of population still have faith in herbal medicine, particularly for chronic diseases and those conditions which are not considered amenable. WHO has estimated that 80% of the world’s populations rely chiefly on traditional medicine. A major part of traditional therapies involve the use of plant extracts or their active constituents. The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethnopharmacologists, Botanists, Microbiologists, and Natural products chemists are combing the earth for phytochemicals and leads which could be developed for treatment of infectious diseases.

3.5.1 Description of some medicinal plants used in this work

a) *Aegle marmelos* Corr (Common name- Bel, Eng.- Bael fruit)

Family - Rutaceae

Description - A small tree, 10m.high; leaves alternate; leaflets 3-5, ovate-lanceolate, flowers 2 sexual, greenish white, 3 cm in diameter, in short panicles, sweet-scented; fruit 5 to 12 cm. in diameter, globose, oblong or pyriform, rind grey or yellow pulp sweet, thick and orange coloured.

Uses - Unripe or half-ripe fruit is used as astringent, digestive, stomachic and in diarrhoea (HMG/N 1993). Fruit is taken in stomachache in Dang districts, Paste of unripe fruit pulp is used by the Mooshars of Dhanusha district to get relief from diarrhoea (Rajbhandari, 2001).

Distribution - Himalayan region of Nepal at 1200m.

b) *Punica granatum* Linn. (Common name- Anar, Eng.- Pomegranate)

Family -Punicaceae

Description - A glabrous shrub of small tree; leaves 2-6 cm.long, oblong or obovate, narrowed to a short petiole; flowers bright red, rarely white of yellowish; fruit ovoid; 5 cm. in diameter and containing much red juice around the seeds.

Uses - Rind of fruit combined with aromatics like cloves, etc.used in diarrhoea and dysentery, Seed as stomachic, Bark used as a vermifuge and in the treatment of diarrohoea and dysentery (HMG/N 1993).

Distribution - Cultivated and some wild forms are also met with in western Nepal.

c) *Psidium guajava* Linn. (Common name- Amba, Eng.- Guava)

Family - Myrtaceae

Description - A small tree; leaves very short petioled, ovate or oblong, 7-10cm. long; flowers 1-3, peduncled, axillary; fruit globose or pear-shaped.

Uses - Leaves used as astringent for bowels, wounds and ulcers; their decoction used in cholera and for arresting vomiting and diarrhoea (HMG/N 1993).

Distribution - Cultivated throughout Nepal.

d) *Acorus calamus* Linn. (Common name- Bojho Eng.- Sweet Flag Rhizome)

Family - Araceae

Description - An aromatic, erect, perennial herb; root stock nerves parallel, spathe leaf-like; spadix 4-8cm., tapering, covered with small, yellow-green, 2 sexual flowers.

Uses - Rhizome-emetic, stomachic, in dyspepsia, colic, remittent fevers, nerve tonic, in bronchitis, dysentery of children and chronic diarrhoea. (HMG/N 1993). Rhizomes are chewed to obtain relief from cough, colds and also from diarrhoea at Palpa districts. (Rajbhandari, 2001).

Distribution - Himalayan region of Nepal at 1800m.

e) *Syzygium cumini* Linn. (Common name- Jamun Eng.- Black plum)

Family - Myrtaceae

Description - A tree 9 m. high; leaves opposite, petiolate, 5.4-17.3 x 2.5-6 cm; ovate, smooth, entire, glabrous, acute; flowers in cyme, white.

Uses - Bark used in the preparation of astringent decoctions; gargles and washes; fresh juice given with goat's milk in the diarrhoea of children. Juice of leaves used in dysentery and juice of ripe fruit made into vinegar used as a stomachic, carminative and as diuretic. Fruit is useful astringent in bilious diarrhoea.

Distribution - Tropical and sub-tropical regions from 1220-1520 m

f) *Anethum sowa* Kurz (Common name- soya Eng.- Dill)

Family - Umbelliferae

Description - Perennial, glabrous or pubescent herb; leaves 2-3 pinnate, leaflets lanceolate or ovate, or entire; flowers white of yellow, in compound umbel; fruit broad winged, 4 x 2mm. and narrowly winged, plano-convex, 2-3 times as broad as thick.

Uses - Fruit used as Carminative and stomachic. Essential oil from the seeds are carminative, useful in flatulence of children

g) *Mimosa pudica* (Common name- Lazzabati, Eng.- Sensitive plant)

Family - Leguminosae

Description - A widely spreading diffused under-shrub; leaves very sensitive, leaflets 12-29 pairs, oblique. Narrow-oblong, acute; flowers in small peduncled heads, pink.

Uses - Decoction of root is useful in gravellish complaints, leaves rubbed into a paste is applied to hydrocele. Leaves and root used in piles and fistula. (HMG/N,1993). Roots are used in diarrhoea, dysentery, cuts wounds (Oli, 2000).

Distribution - Terai regions of Nepal.

h) *Woodfordia fruticosa* Kurz (Common name- Dhayaro, Eng.- Fire flame bush)

Family - Lytharaceae

Description - A pubscent shrub, leaves opposite, sometimes in whorls of three, sessile, lanceolate, 5-10 cm long, entire, under surface white and withblack glandular dots, flowers clustered, numerous, shortly stalked and red corolla.

Uses - Dried flowers used as astringent, used in dysentery, menorrhagia, considered safe stimulant in pregnancy (HMG/N 1993).

Distribution - Himalaya and Terai region of Nepal up to 1500m.

3.5.2 Studies on antimicrobial properties of medicinal plants

Anesini and Perez (1993) reported that among 132 boiling water extracts of Argentine folk-medicinal plants, 10 were found effective against *E. coli*. *Punica granatum* fruit pericarp, *Psidium guineense* fruit pericarp, *Lithrea ternifolia* leaves and *Allium sativum* bulbs produced some of the more active extracts.

Perez and Anesini (1994) screened boiling water extracts of 132 samples from 54 plant families, commonly used in Argentine folk medicine for antibacterial activity against *Salmonella typhi*. Twenty four species showed antibacterial activity. *Punica granatum* fruit pericarp and *Rosa borboniana* flowers produced some of the more active extracts. Taking into account the multiple resistance of *Salmonella typhi*, these findings could be useful in the search for new clinically useful antimicrobials.

Taylor (1995) used methanol extracts of 21 selected plant species against 8 strains of bacteria and 5 strains of fungi and reported all 21 of the extracts showed activity against at least 2 bacterial strains, and 20 showed activity against at least 2 fungi.

Omoregbe *et al* (1996) examined the antimicrobial activity of three ethnolic plants extracts- *Momordica charantia, Alstonia boonei* and *Ocimum bacilicum* on pure and viable cultures of *E. coli, Salmonella paratyphi* and *Shigella dysentriae*. Results showed that the extracts from the leaves of the three plants have some antimicrobial properties against the test organisms which suggest their future possible commercial therapeutic use, although this is a preliminary study.

Bhatta (1998) studied the antimicrobial activity of rind of Punica granatum and found that the extract were more effective against 13 out of 14 tested bacteria.

Nascimento *et al* (1999) evaluated the antimicrobial activity of plant extracts and phytochemicals against antibiotic susceptible and resistant microorganisms. The highest antimicrobial potential was observed for the extracts of *Caryophyllus aromaticus* and *Syzygium joabolanum* which inhibited 64.2 and 57.1% of the tested microorganisms, respectively, with higher activity against antibiotic resistant bacteria (83.3%). The result obtained with *Pseudomonas aeruginosa* was particularly interesting, since it was inhibited by clove, jambolan, pomegranate and thyme extracts.

Baidya (2001) tested the crude ethanolic extract of 20 medicinal plants *Acacia catechu, Acorus calamus, Adhatoda vasica, Aegle marmelos, Artemisia indica, Aspargus racemosus, Bombax malabaricum, Hippophae salicifolia, Holarrhena antidysenterica, Mahonia nepalensis, Mangifera indica, Mentha arvensis, Phyllanthus emblica, Pterocarpus marsupium, Rubus ellipticus, Schima wallichii, Syzygium cumini, Terminalia bellirica, Tinospora cordifolia* and *Zanthoxylum armatum* for antimicrobial activity against *Enterococcus faecalis* (ATCC 29212), *E. coli* (ATCC 25922), Methicillin resistant *Staphylococcus aureus* (MRSA), *Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus* (ATCC 29213).

Prashanth (2001) tested petroleum ether, chloroform, methanol and water extracts of *Punica granatum* for their antibacterial activity. The methanolic extract was found to be most effective against all tested microorganisms.

Prasai (2002) tested 8 different medicinal plants against 7 different gram negative bacteria. Among 8 plants tested, 4 plants viz. *Alnus nepalesnsis, Ficus religiosa, Myrica esculenta* and *Rhododendron arboreum* were found to have effective against *E. coli, Klebsiella, Proteus vulgaris, Salmonella typhi, Shigella* spp. and *Vibrio cholerae.* All the plants were ineffective against *Pseudomonas aeruginosa*. The largest zone of inhibition was obtained with *Rhododendron arboreum* against *Vibrio cholerae* (19mm) and smallest minimum bactericidal concentration value of 6.25 mg/l was obtained with *Alnus nepalesnsis* and *Myrica esculenta* against *Salmonella typhi.*

Rani and Khullar (2003) reported that fifty four plant extracts (methanol and aqueous) were assayed for their activity against multi-drug resistant *Salmonella typhi*. Strong antibacterial activity was shown by the methanol extracts of *Aegle marmelos, Salmalia malabarica, Punica granatum, Myristica fragrans, Holarrhena antidysenterica, Terminalia arjuna* and Triphal (mixture of *Emblica officinalis, Terminalia chebula* and *Terminalia belerica*). Moderate antimicrobial activity was shown by *Picorhiza kurroa, Acacia catechu, Acacia nilotica, Cichorium intybus, Embelia ribes, Solanum nigrum, Carum copticum, Apium graveolens, Ocimum sanctum, Peucedanum graveolens* and *Butea monosperma*.

Timsina (2003) selected 20 medicinal plants and evaluated their antimicrobial activities against 10 test microorganisms including bacteria and fungi. The result showed that 7 (20%) were active against 4 test microorganisms. Gram positive bacteria were more sensitive to medicinal plant extracts than gram negative bacteria. *Staphylococcus aureus* was the most susceptible among 10 test microorganisms. It was inhibited by 19 of 20 plant extracts tested. *Shigella dysenteriae* was found to be the most resistant species being susceptible only to 3 plant extracts.

Agunu *et al* (2005) investigated five medicinal plants [*Acacia nilotica*, *Acanthospermun hispidum*, *Gmelina arborea*, *Parkia biglobosa* and *Vitex doniana*] used in diarrhoeal treatment in Kaduna State, Nigeria. The results obtained revealed that the aqueous methanol extracts of all the five medicinal plants investigated have pharmacological activity against diarrhoea. This may explain their use in traditional medicine for the treatment of diarrhoea.

Alanis *et al* (2005) studied antibacterial properties of aqueous and methanolic extracts of 26 medicinal plants used in Mexico to treat gastrointestinal disorders against eight different species of enteropathogens: two *E. coli*; two *Shigella sonnei* species; two *Shigella flexneri*; and two *Salmonella* spp.. The results showed that all crude extracts exhibited antibacterial activity, at least against one of the microorganisms tested, at concentrations of 8 mg/mL or lower. *Punica granatum* possessed strong antibacterial activity against most of the pathogens tested. In general, methanolic extracts were more active than aqueous extracts.

Mazumder *et al* (2005) studied antidiarrhoeal potential of chloroform extract of the root of *Aegle marmelos* (Correa) Linn. And Of the 35 tested pathogenic diarrhoea causing strains, the extract was found to be mostly active against the strains *of Vibrio cholerae*, followed by *E. coli* and *Shigella* spp.

In Limpopo Province, South Africa, Mathabe *et al* (2006) conducted ethnobotanical survey in which 21 plant species belonging to 14 families were used for the treatment of diarrhoea. Methanol, ethanol, acetone and hot water extract from different plant parts (leaves, roots, bark and stem rhizome), of several plants were screened for antibacterial activity against *Vibrio cholera*, *E. coli* and *Staphylococcus aureus,* Shigella spp., *Salmonella typhi*. Most of the extracts showed relatively high antibacterial activity against most of the tested microorganisms with the diameter of inhibition zones ranging between 10 and 31 mm. Of the plants studied, the most active extracts were those obtained from *Punica granatum* and *Indigofera daleoides*. All extracts from two plants, namely, *Punica granatum* and *Ozoroa insignis* were active against all bacterial strains. Water extract of *Punica granatum* were equally active as organic extracts against bacteria such as *Staphylococcus aureus*, *Shigella sonnei* and *Shigella flexneri*.

Palombo (2006) reveled that of the numerous phytochemicals (such as alkaloids, tannins, flavonoids and terpenes) present in active extracts, tannins and flavonoids are thought to be responsible for antidiarrhoeal activity by increasing colonic water and electrolyte reabsorption.

3.6 Screening of antimicrobial activity

First step of assessment of new antibiotic is screening step and in this step their effectiveness is evaluated this is achieved by determination of zone of inhibition (ZOI), minimum inhibitory concentration (MIC) and/or minimum bactericidal concentration (MBC) for bacteria and minimum fungicidal concentration (MFC) for fungi (Carpinella *et al*, 1999).
Minimum inhibitory concentration (MIC) is the lowest concentration of antibacterial agent that will inhibit visible growth of an organism after overnight incubation (WHO, 1991). MBC is the lowest concentration of antibacterial that is required to kill the bacteria and hence produces sterile culture.

3.6.1 Agar well diffusion (Cup plate) technique

This method was originally standarised by Dingle *et al* (1953) for the evaluation of enzymatic activity for degradation of pectin and other polysaccharides. Latter this method has been modified for the evaluation of antimicrobial activity of the drugs. In this method the agar is inoculated with the test organism and the test solution of antimicrobial or formulations are placed in cups (well) cut out of agar with a sterile cork borer. The zone of inhibition is noted after appropriate incubation (Hugo and Russel, 1984).

3.6.2 Two-fold serial dilution method

This technique has been recommended by WHO (1991) for quantitative estimation of antibiotic activity, in which graded doses of the test substance are incorporated into the broth and the tubes inoculated with the test organism. The point at which no growth occurs during overnight incubation is taken as the minimum inhibitory concentration (MIC), if required the minimum bactericidal concentration (MBC) can be determined by subculturing the last tube to show a visible growth and all the tubes in which thee is no growth. The MBC is the lowest concentration of antimicrobial agent required to produce sterile culture (Cheesbrough, 1993).

CHAPTER-IV

4 MATERIALS AND METHODS

4.1 Materials

A list of materials, chemicals, equipments, media and reagents required for the study is presented in Appendix 1.

4.2 Methods

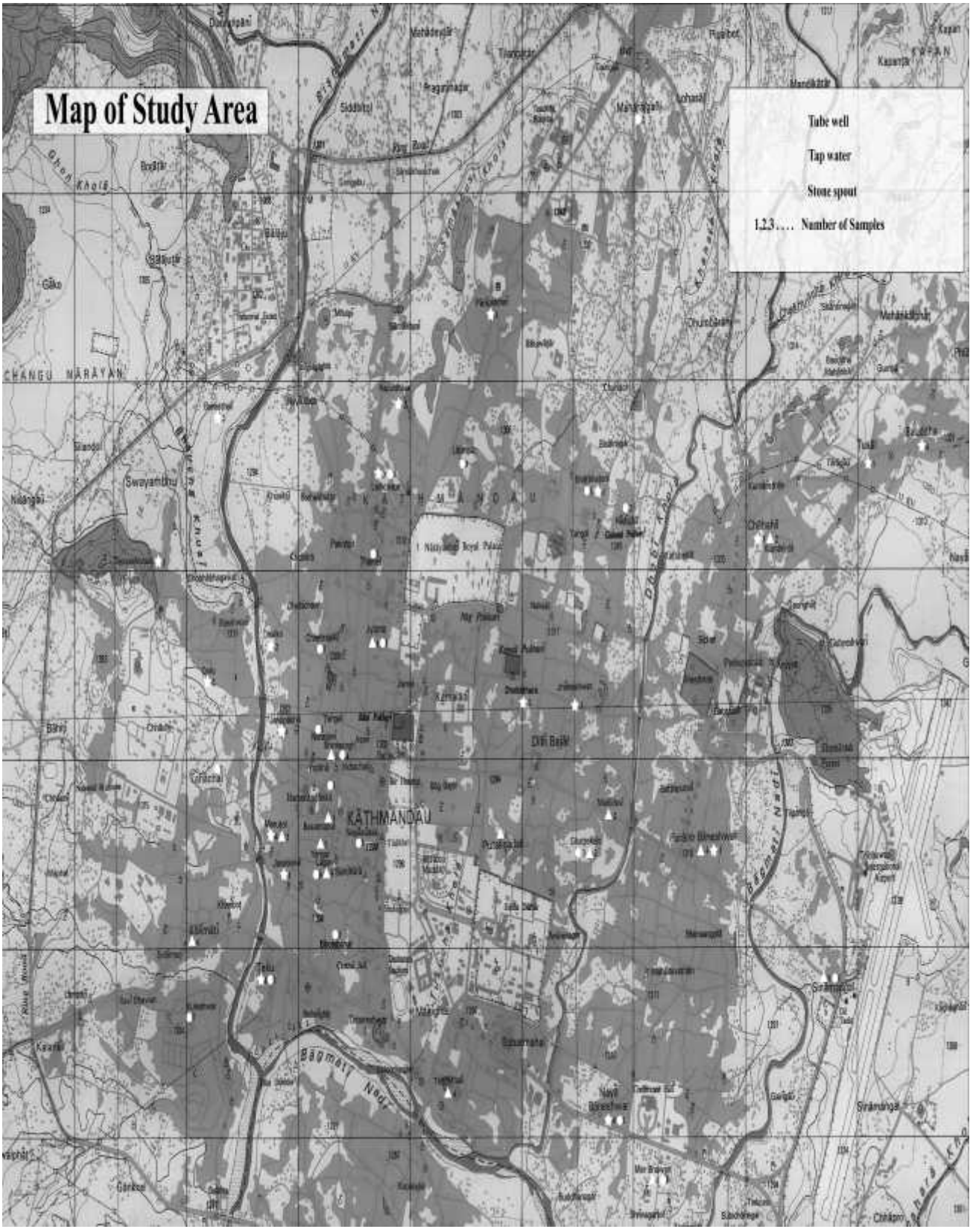
For this study, water samples were collected randomly from three different sources namely tap water, stone spouts and tube wells. The study period was from October 2005 to November 2006.

4.2.1 Study area

This study was conducted in Kathmandu Metropolitan City (KMC) area of Kathmandu district. Kathmandu, the capital, lies in Bagmati zone of the Central Development Region of Nepal.

In Kathmandu district, there are 57 VDCs and two municipalities (Kathmandu Metropolitan City and Kirtipur Municipality), Kathmandu being the Capital city as its headquarters. According to 2001 census, the total population of this district was 1,081,845 with average household size 4.60 and annual growth rate (1991-2001) of 4.82%. By caste/ethnicity majority of the people are Newar followed by Brahmin, Chhetri and Tamang. Population with access to safe drinking water accounted 84.15% (ISRSC, 2002).

Administratively, KMC consists 35 wards. According to 2001 census, the total population of KMC was 671,846 with average household size 4.42 (1991-2001). By caste/ethnicity, Newar people are the native and dominant population.


Map of Study Area
Tube well
Tap water
Stone spout
1,2,3.... Number of Samples
KATHMANDU
Bagmati Nadi
CHANGU NARAYAN
Swayambhu

4.2.2 Collection of samples for water analysis

Water samples for bacteriological testing were collected in sterile bottles and care must be taken to prevent accidental contamination of the water during its collection. Glass bottles used for water sampling had capacity of at least 200 ml. It was fitted with ground glass stoppers or screw caps. The stopper or cap and neck of the bottle were protected from contamination by a suitable cover either of paper or thin aluminium foil. Silicon rubber liners, that withstand repeated sterilization at 160^0C, were used inside screw caps. After sterilization, the bottle was not opened before the sample was collected. Samples were collected according to Cheesbrough (2000).

4.2.2.1 Sample collection

The sterile bottle was held by the base in one hand. The other hand was used to remove the stopper and covered together. The stopper and cover were retained in the hand while the bottle was filled and then replaced together. Contamination was prevented during sampling and water was not touched. Contaminated bottle was not used. A total of one hundred and fourteen samples of drinking water were randomly collected from different place of Kathmandu Valley.

4.2.2.1.1 Sample collection from tap

1. Any external fittings from the tap were removed, such as an antisplash nozzle or rubber tube.
2. Outside nozzle of the tap was cleaned carefully, especially any grease, which has been collected.
3. The tap was turned on full, and the water was allowed to run to waste for 1 minute in order to flush the tap and discharge any stagnant water.
4. The tap was sterilized by igniting a piece of cotton-wool soaked in methylated spirit and holding it with a pair of tongs closed to the nozzle until the whole tap was unbearably hot to touch.
5. The tap was allowed to cool by running the water to waste for a few seconds.
6. The sample bottle was filled from a gentle flow of water, and replaced cap of the bottle.

7. The sample bottle was labelled with the sample code number immediately after filling it with the water sample.

Sampling Sites:-

S.no.	Lab code	Location/ward no
1	TP01	House no 88,Karunamaya Marg, Lagan tole -21
2	TP02	House no 47, Wanta Baha, Lagan tole-21
3	TP03	House no 21/19, Kissigalli, Bhedasingh-18
4	TP04	House no 147, Ikubaha, Yangal-21
5	TP05	House no 83, Ikubaha, Yangal-21
6	TP06	House no 45, Karunamaya Marg, Lagan tole-21
7	TP07	House no 23/21 Punchhe Galli, Ombahal-23
8	TP08	House no 87/23Ga, Chikanmuga galli, Chikanmugal-20
9	TP09	House no 160/33,Talachhi Marg, Khabahal,Jyatha-30
10	TP10	Sugam Marg, Chabahil-7
11	TP11	House no 30, Padmasugandha Marg, Yangal-21
12	TP12	House no 180, HridayaChandraSingh Marg, Marudhoka-19
13	TP13	House no 649/45, Layaku, Basantapur-19
14	TP14	House no 249, Nyapacho Marg, Lagan tole-21
15	TP15	House no 74, Ashokbinayak Marg, Maru-20
16	TP16	House no 247, Swetbinayak Marg, Thapathali-11
17	TP17	House no 303, Prashuti Marg, Thapathali-11
18	TP18	House no 40, Rajesh Marg, Thapathali-11
19	TP19	House no 219/22, Radhakrishna Marg, Thapathali-11
20	TP20	House no 89, Karunamaya Marg, Lagan tole-21
21	TP21	House no 62/50, Tulsi Marg, Ghattekulo-32
22	TP22	House no 489, Birendra Marg, Maitdevi-32
23	TP23	House no 485, Birendra Marg, Maitdevi-32
24	TP24	House no 104, ShreeRam Marg, Puranobaneswor-9
25	TP25	House no 102Kha, ShreeRam Marg, Puranobaneswor-9
26	TP26	House no 102Ka, ShreeRam Marg, Puranobaneswor-9
27	TP27	Omnagar, Sinamangal-9
28	TP28	Nakhya Galli, Teku-12
29	TP29	Club Marg, Kalimati,-13
30	TP30	Club Marg, Kalimati-13
31	TP31	House no 310, Ganesh Man Singh Path, Kalimati Chok-13
32	TP32	House no 359, Ganesh Man Singh Path, Kalimati Chok-13
33	TP33	House no 62/50, Tulsi Marg, Ghattekulo-32
34	TP34	House no 204/15, Padmasugandha Marg, Mazipat-21
35	TP35	House no 39, Khusilappu Marg, Putalisadak-31
36	TP36	Chandra Binayak Marg,Chabahil-7
37	TP37	ShreeRam Marg, Purano Baneswor-9
38	TP38	Minbhawan marg, Minbhawan-34

4.2.2.1.2 Sample collection from tube well

1. The hand pump was operated continuously for five minutes.
2. The mouth of pump was heated, preferably by means of a spirit lamp, and pumped several gallons of water to waste.
3. Sample was collected aseptically by allowing the water from the pump to flow directly into the sterile bottle and replaced the bottle cap carefully.
4. The bottle was labelled with the sample code number.

Sampling Sites:-

S.no.	Lab Code	Location/Ward no
1	TW01	Talachhi Marg, Jyatha-30
2	TW02	Jamna Gubhaju Marg, Jyabahal-21
3	TW03	Karunamaya Marg, Lagan Tole-21
4	TW04	Karunamaya Marg, Lagan Tole-21
5	TW05	Jamna Gubhaju Marg, Jyabahal-21
6	TW06	NKSingh Marg, Minbhawan-34
7	TW07	Bogati Marg,New Baneswor-10
8	TW08	Nakhya Galli, Teku-12
9	TW09	Kaskeli galli, Lainchour-29
10	TW10	Jamna Gubhaju Marg, Jyabahal-21
11	TW11	Shree Kumari Marg, Bhatbhateni-5
12	TW12	Bhimsen Marg, Bhatbhateni-5
13	TW13	Pachhe Galli, Nardevi-18
14	TW14	Maharajgung-3
15	TW15	Pashikeba Marg, New Road-24
16	TW16	Ratnakirti Mahabir, Makhan-25
17	TW17	Om Nagar Marg, Sinamangal-9
18	TW18	Paknajol Marg, Chhetrapati-16
19	TW19	Karunamaya Marg, Lagan Tole-21
20	TW20	Maharajgung-3
21	TW21	Kotal marg, Hadi Gaun-5
22	TW22	Tulsi Marg, Ghattekulo-32
23	TW23	Karunamaya Marg, Lagan Tole-21
24	TW24	Karunamaya Marg, Lagan Tole-21
25	TW25	Tulsi Marg, Ghattekulo-5
26	TW26	Tulsi Marg, Ghattekulo-5
27	TW27	Kissigalli, Bhedasingh-18
28	TW28	Kissigalli, Bhedasingh-18
29	TW29	Kissigalli, Bhedasingh-18
30	TW30	Kissigalli, Bhedasingh-18
31	TW31	Kuleshwore-14

S.no.	Lab Code	Location/Ward no
32	TW32	Jamna Gubhaju Marg, Ikubahal -21
33	TW33	Ghattekulo-32
34	TW34	Mueseum Marg,Tahachal-13
35	TW35	Thamel Marg, Thamel-29
36	TW36	Bhotebahal Marg, Bhotebahal-22
37	TW37	Karunamaya Marg, Lagan Tole-21
38	TW38	Purano Dhungedhara Marg, Panipokhari-3

4.2.2.1.3 Sample collection from stone spouts

1. The bottle was held by the base in one hand while the other hand was used to remove and replace the screw cap or the stopper.
2. Water was collected facing the mouth of the bottle towards the opposite direction of the water current.
3. The neck of the bottle was slightly tilted upwards to let it fill completely before carefully replacing the cap.
4. The bottle was labelled with the sample code number.

Sampling Sites:-

S.no.	Lab Code	Stone spout	Location
1	SS01	Maru hiti	Ashok Binayak Marg, Maru-20
2	SS02	Kohiti	Kohiti Marg, Jaisidewal-19
3	SS03	Vanjha hiti	Hridaya Chandra Singh Marg, Maru-19
4	SS04	Bhindio hiti	Paropakar Marg, Bhimsensthan-20
5	SS05	Chasan hiti	Chasando Galli, Maru Dhoka-19
6	SS06	Dhunge dhara	Taha Galli, Dhoka tole-19
7	SS07	Tulsi dhara	Amar Chitrakar Marg, Dallu-15
8	SS08	Chhahare dhara	Chhahare Galli, Dallu-15
9	SS09	Dhunge dhara	Ajima Galli, Bhatbhateni-5
10	SS10	Dhunge dhara	Mahakabi Marg, Gyaneswor-33
11	SS11	Dhobidhara 1	Dhobidhara Marg, Dhobidhara-33
12	SS12	Dhobidhara 2	Dhobidhara Marg, Dhobidhara-33
13	SS13	Tadha Hiti 1	Swayambhu Marg, Dhalko-17
14	SS14	Tadha Hiti 2	Rajak Galli, Dhalko-17
15	SS15	Kapoor Dhara 1	Kapoordhara Marg, Kapoordhara-29
16	SS16	Kapoor Dhara 2	Kapoordhara Marg, Kapoordhara-29
17	SS17	Dhunge dhara	Purano Dhungedhara Marg, Panipokhari-3
18	SS18	Dhunge dhara	Pooja Pratisthan Marg, Baneswor Height-10
19	SS19	Dhunge dhara	Hanuman Chok Marg, Lainchaur-29
20	SS20	Gahiti 1	Gahiti Marg, Lainchaur-29
21	SS21	Gahiti 2	Gahiti Marg, Lainchaur-29

S.no.	Lab Code	Stone spout	Location
22	SS22	Dhungedhara	Subarna Marg, New Baneswor-10
23	SS23	Dhungedhara	Pandhero Galli, New Baneswor-10
24	SS24	Dhungedhara	Sajha Galli, New Baneswor-10
25	SS25	Angat Dhara	Angatdhara Marg, Tushal-6
26	SS26	Ram Hiti	Siromani Marg, Ramhiti, Bouddha-6
27	SS27	Dhungedhara	Dharatole, Bouddha-6
28	SS28	Dhungedhara	Dharatole, Bouddha-6
29	SS29	Dhungedhara	Bouddha phoolbari Marg, Bouddha-6
30	SS30	Pinas Dhara	New Baneswor-10
31	SS31	Butha hiti	Siddhi Charan Sadak,Banashthali Chok-16
32	SS32	Gahiti	Yog Bir Singh Marg, Tamsipakha-18
33	SS33	Erahiti	Era Hiti Marg, Chakrapath-16
34	SS34	Dhunedhara	Swayambhu Parikrama Marg, Swayambhu-15
35	SS35	Gairi dhara	Gairidhara Marg, Gairidhara-1
36	SS36	Dhungedhara	Hospital Marg, Chabahil-7
37	SS37	Dhunedhara	Dathu tole, Jayabageswori-8
38	SS38	Dhunedhara	Gaurighat Marg, Chabahil-7

4.2.2.2 Transportation and preservation of sample

The collected water samples were analyzed in Environment Laboratory of NAST, Khumaltar on the same day immediately after its delivery and always within 6 hours of collection. In some cases when immediate analysis was not possible, the samples were preserved at 4^0C.

For determination of arsenic and iron contents, 2 ml concentrated hydrochloric acid was kept in the sample bottles before the collection of water sample in order to preserve the sample in reduced state.

4.2.2.3 Water source information

In present study, general information of water sources (name of locality, ward number, source type, owner's name etc) was collected by interviewing a household head, knowledgeable family member or other knowledgeable person using the source.

4.2.2.4 Water test analysis

Basically two types of parameters viz. physicochemical and microbial were analyzed for assessment of drinking water quality.

4.2.2.4.1 Study of physico-chemical parameters of water samples

Analysis of most of the physico-chemical parameters of water were done by following "Standard Methods for the Examination of Water and Wastewater" (APHA, 1998). The temperature and pH of water samples were recorded at the site during sampling period. All other parameters were analyzed in the Environment laboratory of NAST.

A. Temperature

Temperature was determined with the help of a standard mercury thermometer graduated up to 50^0C. Soon after collection of the sample, thermometer bulb was immersed into the water and noted the reading.

B. pH

Hydrogen ion concentration in the sample was measured with the help of a pH meter (TOA Electronics, Japan) by inserting the electrode into the water sample.

C. Conductivity

Conductivity was measured with the help of a conductivity meter (LF 91). The electrode was first rinsed with distilled water and then immersed into water sample, gently stirred and noted the reading.

D. Turbidity

The turbidity of sample was measured with the help of a Nephelometer (Elico, India). The sample was put in the clean, free of any scratch sample tube taking a reference with standard turbidity suspension and noted the reading on the scale.

E. Total hardness

The total hardness of the water sample was determined by EDTA titrimetric method. For this 50 ml of sample was taken in a clean conical flask to which 1ml of ammonia buffer solution was added and stirred for thorough mixing. Then 200 mg of Erichrome Black T indicator was added and shaken well. The contents in the flask was titrated against standard EDTA solution (0.01M) with continuous stirring, until the colour changed from wine red to blue at the end point. The volume of EDTA consumed was noted and total hardness was calculated by following equation (APHA, 1998).

$$\text{Total Hardness as mg/l } CaCO_3 = \frac{\text{ml EDTA consumed} \times 1000}{\text{ml of sample}}$$

F. Iron

Iron contained in the water sample was determined by Phenanthroline method as described in APHA, (1998). For this, 50 ml sample was taken in 100ml conical flask to which 2ml conc. HCl and 1ml of 10% hydroxylamine hydrochloride ($NH_20H.HCl$) solution were added with separate clean pipettes The contents in the flask was boiled to half of the volume for dissolution of all the iron and then cooled to room temperature and transferred to a 100-ml volumetric flask. Then, 10ml ammonium acetate ($CH_3COONH_{4)}$ buffer solution and 2ml 0.1% phenanthroline solution were added which forms orange-red colour. It was diluted to 100 ml with distilled water, mixed thoroughly and allowed to stand at least 10 to 15 minute for maximum colour development. The absorbance was read at 510nm on a spectrophotometer in which distilled water served as a blank. The concentration of total iron in the sample was determined by correlating it with the absorbance in the standard calibration curve prepared by using various dilutions of standard iron solution (1 to 4 mg/l).

G. Arsenic

The total arsenic in water was determined using quantofix arsenic test kit. This kit has capability of detecting arsenic concentration from 0.01mg/l to 0.5 mg/l.

Method of application was as described in kit leaflet.

1. A syringe was used to add 2 × 10ml sample solution into the reaction vessel.
2. Then 1 measuring spoon reagent Arsenic-1 was added and reaction vessel was swirled gently.
3. 1 measuring spoon reagent Arsenic-2 was added.
4. A test stick was taken out from the container and inserted into reaction vessel with test field 2 cm and clamped with lid.
5. For pressure balance the needle was pricked through the lid and left there.
6. During 30 min reaction time, the reaction vessel was swirled 3 times gently. The test field should not get in contact with the sample.
7. After 30 min, test strip was removed from reaction vessel, dipped into water for 2 sec, excess liquid was shaked off and test field was compared to colour scale.

H. Ammonia

Ammonia was determined as described in Macherey-Nagel test kit. This test kit can detect ammonia from 0 to 3 ppm within 5 minutes.

1. The test vessel was rinsed several times with the water sample and filled to the ring mark (5 ml).
2. 10 drops of NH_4-1 was added and mixed by swirling.
3. 1 level measuring spoon of NH_4-2 was added and dissolved by swirling
4. After 5 min, 4 drops of NH_4-3 was added and mixed by swirling.
5. Again after 5 min, the measuring vessel was placed on the colour chart and assigned the value by comparison of the colour. Mid-values can be estimated.

I. Nitrate

Nitrate was determined as described in Macherey-Nagel test kit. The kit detects nitrate at a range of 0 to 50 ppm within 5 minutes.

1. The test vessel was rinsed several times with the water sample and filled to the ring mark (5ml).
2. 5 drops of NO_3-1 was added and mixed by swirling.
3. 1 level measuring spoon of NO_3-2 was added and swirled for 30s.
4. After 5 min, the measuring vessel was placed on the colour chart and assigned the value by comparison of the colour. Mid-values can be estimated.

4.2.2.4.2 Microbial examination of water sample

A. Total coliform count

In this study, total coliforms were enumerated by the membrane filtration (MF) technique as described by APHA (1998).

Standard total coliform membrane filter procedure

1. First of all, sterile filter holder with stopper was assembled on the filter flask.
2. Using sterile blunt-edged forceps, a sterile membrane filter of pore size 0.45 µm (grid side up) was placed over the porous disc in such a way that it overlapped the entire circumference of sintered filterable area.
3. The sterile funnel was securely placed on the filter base.
4. The sample of water was well mixed by inverting the bottle several times, and then 100 ml of the water sample was poured into the funnel.
5. The sample was slowly filtered under partial vacuum by using electric vacuum pump.
6. The funnel was removed and the membrane was directly transferred, keeping its upper side upwards, onto a plate of M-Endo agar with the help of sterile forceps. Care was taken not to entrap air bubbles between the membrane and the medium.
7. Then it was incubated for 24 hrs at 37^0C in inverted position.
8. After proper incubation total colony forming unit (CFU) were counted. For this, all sheen-producing colonies were counted.

B. Detection of *Salmonella* and *Shigella* species

5ml of water sample was enriched by inoculated into 45ml Selenite F broth. It was then incubated at 37°C for overnight, and a loopful of the upper part of the broth was subcultured on a selective enteric medium, SS agar. The plate was incubated at 37°C for 24 hours (Collee *et al*, 1996).

C. Detection of *Vibrio cholera*

1ml of water sample was enriched by inoculated into 9ml of 1 % alkaline peptone water. It was then incubated at 37^0C for 6 to 8 hours. Then a loopful of the enrichment broth was

streaked on TCBS agar medium plate. The pate was incubated at 37^0C for 24 hours (Collee *et al*, 1996).

4.2.2.4.2.1 Isolation and identification of bacteria

Sheen producing colonies and all colonies with different characteristics from M-Endo agar, SS agar and TCBS agar were streaked onto NA to get pure culture. Bacteria isolated on respective selective or differential media were identified on the basis of their colonial characteristic, morphological characteristics and biochemical properties. Identification was carried out following Bergey's Manual of Systematic Bacteriology, 1994. Biochemical tests for identification of isolates were given in Appendix 2.

4.2.3 Antibacterial properties of medicinal plants

4.2.3.1 Selection of medicinal plants

Eight different medicinal plants were selected on the basis of magnitude of their use among the different ethnic groups of Nepal and their use for common disorder. Only those medicinal plants were selected which were found to be useful for different common diseases like diarrhoea, dysentery, fever etc.

4.2.3.2 Collection of medicinal plants

Different parts of selected medicinal plants were collected from different parts of Kathmandu Valley. The list of medicinal plants, their corresponding part used in this study, month of sample collection and location/place of the sample plants was given in the Appendix 3.

4.2.3.3 Processing of the samples

A. Washing and chopping

Bark, root and other underground parts were washed thoroughly to remove soil and extraneous matter such as other parts of the same plant or grasses with herbs or any other unwanted matter in case of rhizome also removed. Collected samples were then chopped into small fragments. Stems, roots, twig and bark were chopped into 3-5 cm pieces and split longitudinally into several sections.

B. Shade drying of sample

All samples collected from the spot were subjected for shade drying separately in blotting paper in dark room till they became dry.

C. Packaging and storage of the samples

The completely dried plant matter was packed in waterproof bags and incompletely dried samples (if any) were kept in cotton bags which enhanced the air circulation and hence, prevent rotting during storage. The packed samples were stored in room temperature away from direct sunlight.

D. Grinding the samples

The dried samples were cut to small pieces by means of plant cutter and they were subjected to grinding using grinder to obtain fine powder.

4.2.3.4 Soxhlet extraction with dehydrated ethanol

The extraction process was carried out in Natural Product Research Laboratory, NAST. Dried plant powder was weighed and a known weight was loaded in a clean and dried thimble of the soxhlet extractor. It was then fitted with appropriate sized pre-weighed, dried and properly labeled round bottom flask having a capacity of 250 ml. It was set up with the help of stands. Then 150 ml of ethanol was slowly poured from upper mouth of the soxhlet extractor. It was then fitted with condenser. The flask was heated with heating mantle. The solvent vapours reached the cylinder through the side tube and passed into the condenser. The condensed solvent dropped on the powder of medicinal plant and dissolved soluble compounds. The solution filters and passed into the cylinder and the dissolved soluble compounds flows back to the flask (Tewari *et al,* 1992). The process was allowed to run for 8-15 hours or till the coloured solvent appeared in siphon.

A. Removal of solvent

After complete extraction, the round bottom flask containing extract was fitted with rotatory vacuum evaporator under negative pressure. The flask was constantly heated in rotating

condition by using water bath below 55^0C. Solvent was completely removed and was collected in a separate round bottom flask and later it was collected in a sterile bottle. The round bottom flask containing the extract was weighed till constant weight appeared and result was noted. The weight of the pre-weighed round bottom flask was subtracted from the weight of round bottom flask with extract to find out the extract yield. The crude extract was then transferred in a bottle by sterile spatula. It was then labeled and stored in a refrigerator.

B. Preparation of working solution

One-gram crude ethanol extract from each medicinal plant was aseptically transferred into a clean & dried sterile 20 ml screw cap test tube. 9ml of sterile distilled water was added in each test tube & vortex the tubes to make homogenous mixture/solution/suspension of 1g/10ml i.e. 100mg/ml working suspension or solution and stored in a refrigerator (2-8^0C)

4.2.3.5 Maintenance of test organisms

Test organisms were isolated from drinking water source from Kathmandu City. The bacterial cultures were maintained on nutrient agar slants in closed vials. The organisms were sub cultured every two weeks.

A. Preparation of standard culture inoculums

Standard culture inoculums were prepared from primary culture plate as follows. Three to five colonies of similar appearance of the organism to be tested were touched with the inoculating loop aseptically. It was then transferred to a tube containing (5ml) sterile nutrient broth. The tube was then compared with turbidity standard (Appendix 4) as recommended by WHO (1991) for antimicrobial susceptibility test and the density of the test organism suspension was adjusted by adding more bacteria or more sterile distilled water.

4.2.3.6 Qualitative screening and determination of antibacterial activity

The crude extract of medicinal plant was screened for its antibacterial activity i.e. determination of zone of inhibition against tested organism by agar well diffusion method as given by Dingle *et al* (1953). Sterile Mueller-Hinton agar plates were prepared. Before using the plates, they were dried under laminar flow or incubation at 37^0C for 30 minutes to remove

excess of moisture from the surface of media. The fresh bacterial culture comparable with turbidity standard was prepared as in Appendix 4.

Sterile cotton swab was dipped into the prepared inoculums and rotated and pressed against the upper inside wall of the tubes above the liquid culture level to remove excess inoculums and seeded carefully all over the plate. The plate was then rotated through an angle of 60^0C after each swabbing. Finally the swab was then passed round the edge of the agar surface. The inoculated plate was left to dry a few minutes at room temperatures with the lid closed (WHO, 1991).

Then with the help of sterile cork borer no. 6, wells were made in the inoculated media plate and labeled properly with permanent marker pen. The diameters of wells were 6mm. 50µl of the working solution/ suspension of different medicinal plants was transferred into the well with the help of micropipette. The solvent itself was also tested for its antibacterial activity as control at the same time in a separate well. The plates were then left for half and hour with lid closed so that the extract diffused into the media. After diffusion, the inoculated plates were incubated at 37^0C overnight.

After proper (18-24 hours) incubation, the plates were viewed for the zone of inhibition, which is suggested by clear area without growth around the well. The zones of inhibition were measured using a scale and mean was recorded.

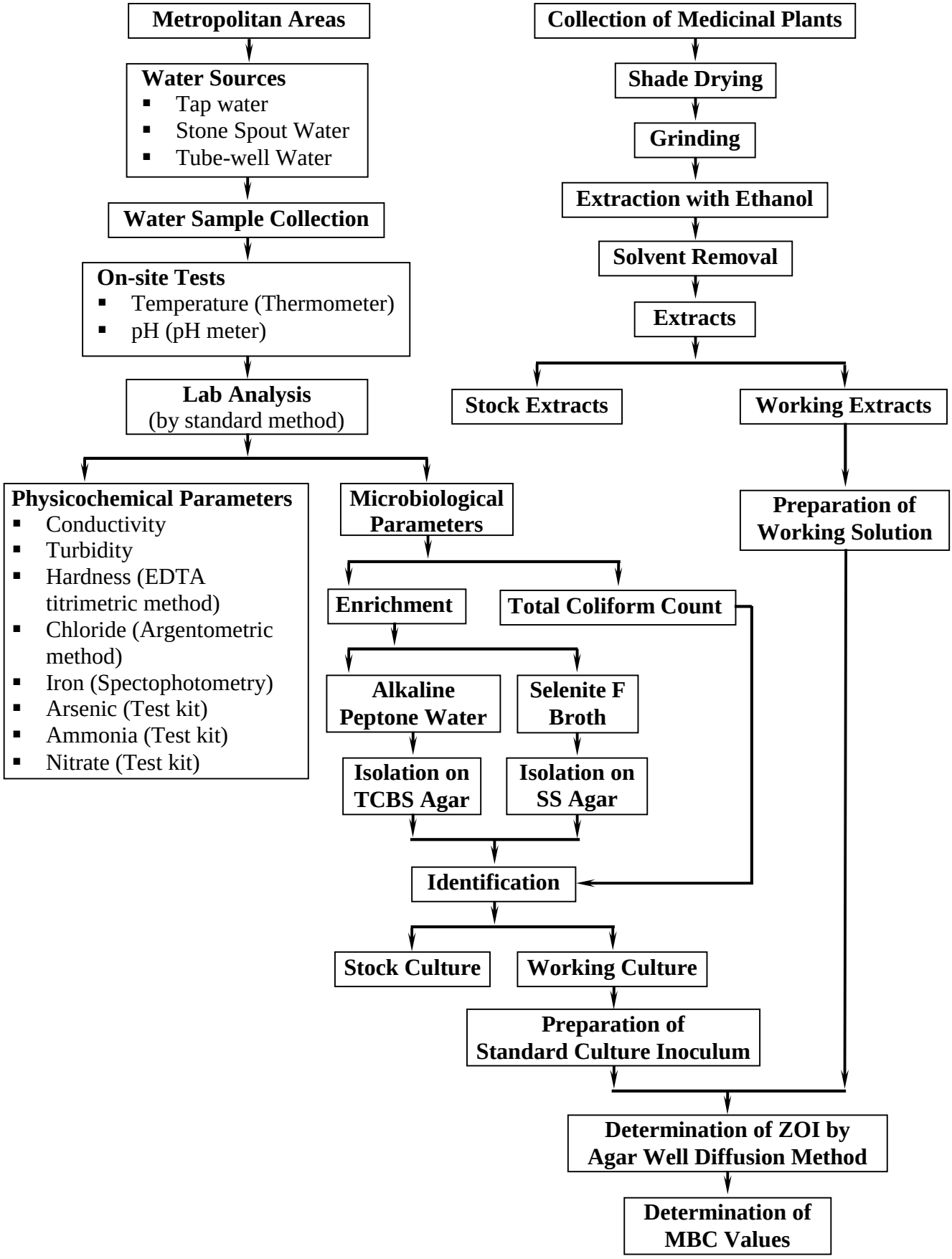
4.2.3.7 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The crude extracts which showed antibacterial activity were subjected to two-fold serial dilution method to determine minimum bactericidal concentration (MBC). For each bacterium, a set of 12 screw-capped test tubes containing 1ml nutrient broth was required. The test tubes were labelled as positive growth control, negative growth control and numbers1 to 10. In case of negative control nutrient broth was discarded. Then 1ml of crude extract from a particular medicinal plant was added aseptically to each tube labeled as negative growth control and 1 no. labelled test tube. The 1^{st} tube now contains 1ml of broth and 1ml of extract. After complete homogenization 1ml of its content was transferred

aseptically to 2nd tube. Similarly the second tube was homogenized and 1ml of its content was transferred to 3rd tube. This process of homogenization followed by transfer was carried up to the 10th tube. Finally after homogenization 1ml of content from the 10th tube was discarded. In this way, two fold serial dilution of the plant extract was prepared upto the 10th tube, each containing equal volume but decreasing concentration.

No plant extract was added to the labeled as positive control. Now with the help of micropipette, 50µl of culture inoculums of test bacteria prepared as mentioned in section 4.2.3.5(A) was added to each tube except the one was labeled as negative control. All the tubes were incubated at 37^0C for 24 hours and observed for turbidity by comparing with +ve and –ve controls. The results were interpreted on the basis of the fact that growth occurs in the positive control and any other tube in which the concentration of the extract is not sufficient to inhibit growth and the lowest concentration of the agent that inhibits growth of the organism as detected by lack of visible turbidity as designated the minimum inhibitory concentration (MIC). However, in some cases it was difficult to identify whether the turbidity was due to the growth of bacteria or due to the turbidity of plant extract itself, the tubes were sub cultured on nutrient agar plate with proper label and incubated at 37^0C for 24 hours. Then they were examined for the growth of bacteria. The tube with minimum concentration of the extract in which the growth was completely stopped was also clearly notified. This determines the minimum bactericidal concentration (MBC); MBC is the lowest concentration of antibacterial that is required to kill the bacteria and hence produces sterile culture.

Flow Chart of the Methods



CHAPTER-V

5 RESULTS

A total of one hundred and fourteen water samples were randomly collected from different places of Kathmandu metropolitan areas. All water samples were analysed for physicochemical and microbiological parameters to assess the drinking water quality. Out of total 114 samples, 38 each were collected from three different sources namely stone spouts, taps and tube wells. Source wise distribution of samples showed the equal number of samples from three different sources.

5.1 Physico-chemical parameters of water

The analysis of physico-chemical parameters of collected water samples was considered as one of important tool for drinking water quality assessment and hence several parameters were tested.

Temperature

The temperature of water samples showed a remarkable variability ranging from 23.2^0C to 30.2^0C, 21.1^0C to 30.1^0C and 18^0C to 30.1^0C for stone spouts, tap water and tube wells respectively. The minimum temperature (18^0C) was recorded in the month of February in tube well water collected from Bhotebahal, while the maximum temperature (30.2^0C) was recorded in the month of June from Tulsidhara, Dallu area. The source wise distribution of average temperature showed the highest figure in case of tap water (26.86^0C) followed by tube wells ($26.52\ ^0$C), and stone spouts (26.51^0C).

Table 1 Source wise distribution of temperature of water samples

S.N	Source	Temperature (°C)		
		Minimum	Maximum	Average
1.	Stone spout	23.20	30.2	26.51
2.	Tap water	21.10	30.1	26.86
3.	Tube well	18.00	30.1	26.52

pH

A notable variation in pH value was observed. The value ranged from 6.1 in tube well water of Bhatbhateni to 8.3 in tap water of Kalimati. A comparision of pH value with WHO standard revealed that 85.09 % of samples were within standard, 14.91% below the standard and 0% above the standard. Comparing to the sources, all the samples from tap were within standard.

Table 2 Source wise distribution of pH values of water samples

S.N	Source	pH value		
		Minimum	Maximum	Average
1.	Stone spout	6.2	8.2	6.83
2.	Tap water	6.5	8.3	7.07
3.	Tube well	6.1	8.0	6.71

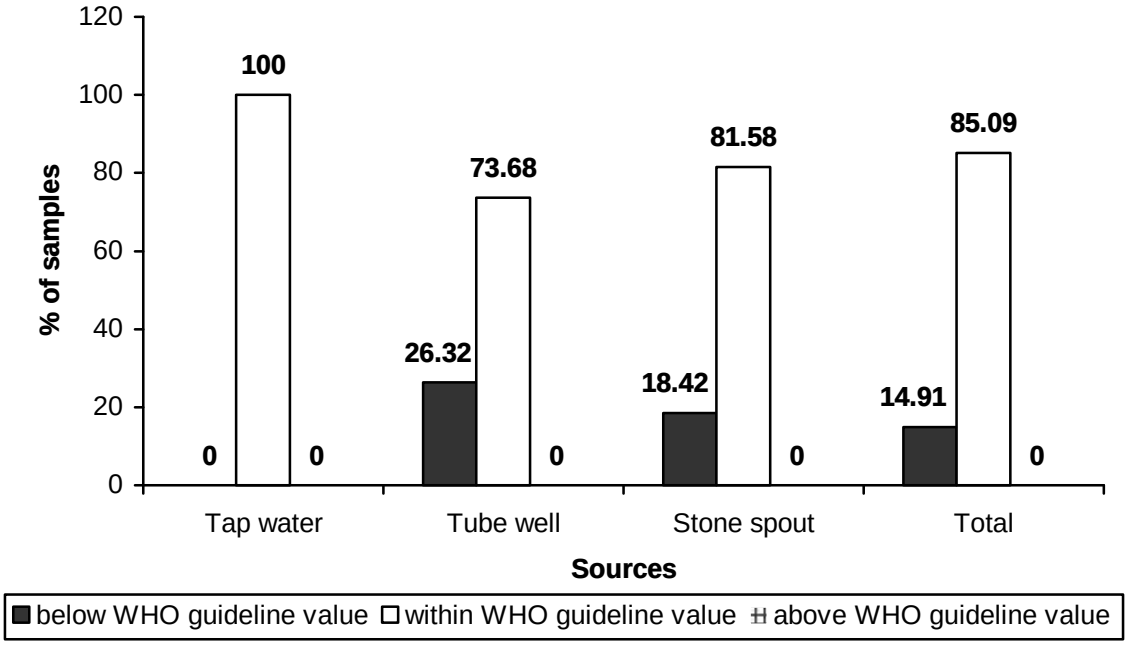


Figure 3 Comparision of pH measurements of water samples with standards

Conductivity

Conductivity was another physical parameter in which notable variability was experienced. The electrical conductivity measurement values ranged from 21 µs/cm in stone spout water sample of Boudha to 1130 µS/cm in tube well water of Lagan tole. A comparison with permissible level showed that majority of water samples (75.44%) remained within limit set by WHO. However, 24.56% samples crossed the limit.

Table 3 Sourcewise distribution of Conductivity Measurements of Water Samples

S.no.	Source	Conductivity (µs/cm)		
		Minimum	Maximum	Average
1	Stone Spouts	21.00	879.00	376.05
2	Tap Water	31.00	747.00	132.37
3	Tube Well	187.00	1130.00	482.82

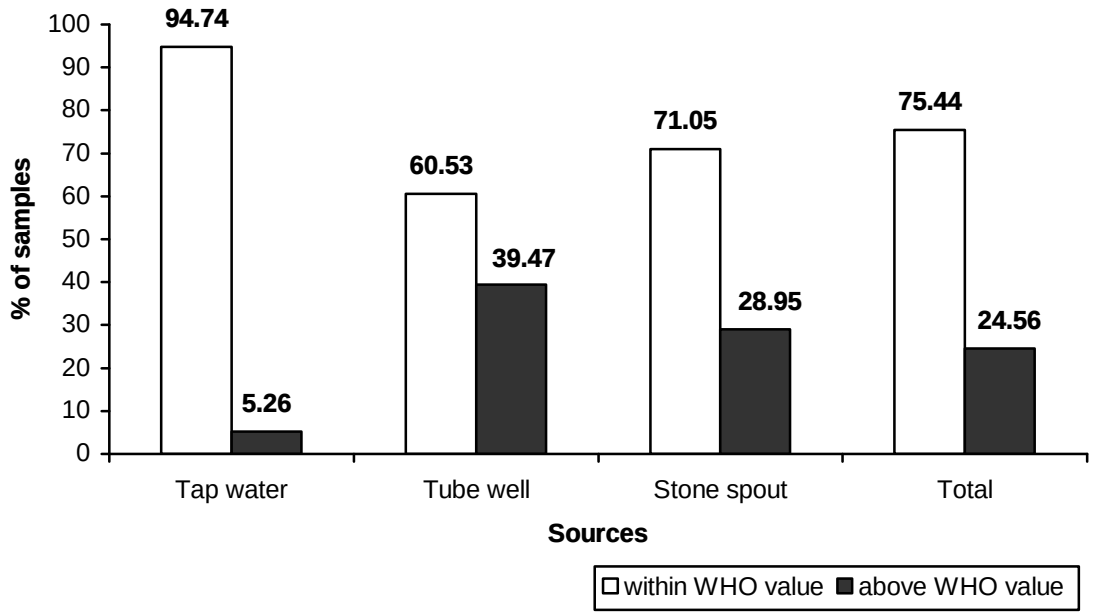


Figure 4 Comparison of conductivity measurements of water samples with standards

Turbidity

The turbidity measurement of analysed water samples also showed a remarkable variability ranging from minimum 0 NTU in many samples of all sources to maximum 770 NTU in tube well water collected at Bhotebahal. Source wise turbidity measurement revealed that tap water were highly turbid whereas waters collected from stone spouts were least turbid. The comparison test results of turbidity measurement showed most water samples, 26.32%, crossed the permissible guideline value as prescribed by WHO.

Table 4 Source wise distribution of turbidity measurements of water samples

S.no.	Source	Turbidity (NTU)		
		Minimum	Maximum	Average
1	Stone Spouts	0.00	13.20	1.29
2	Tap Water	0.00	48.00	5.98
3	Tube Well	0.00	770.00	27.19

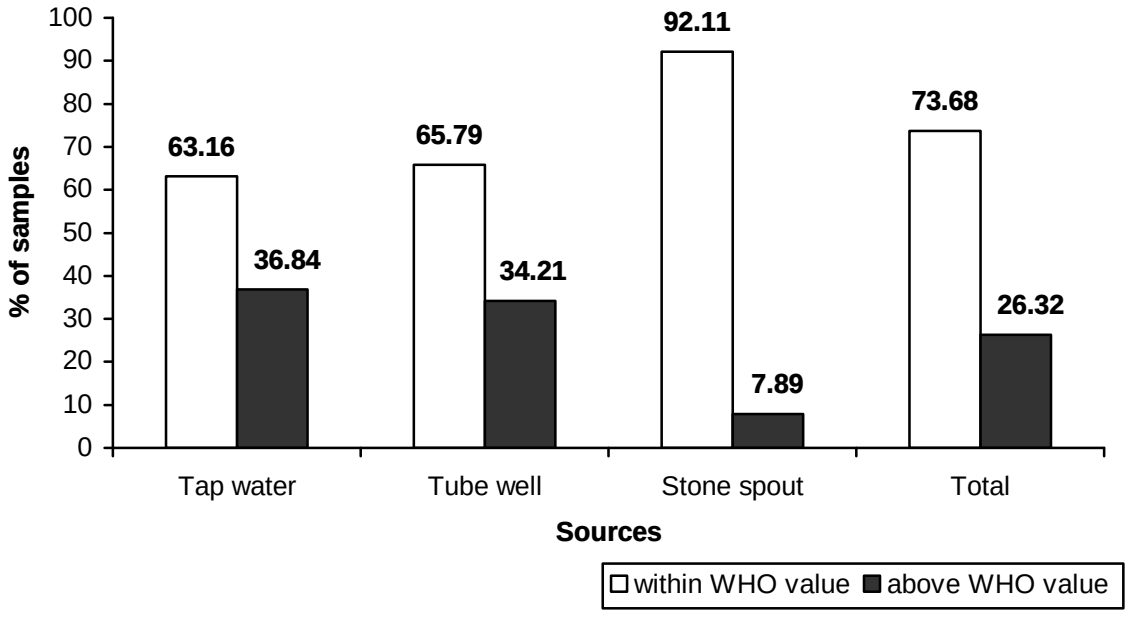


Figure 5 Comparison of turbidity measurements of water samples with standard

Hardness

A notable variation in hardness of tested water samples was recorded. The minimum hardness 8 mg/l was recorded from tap water at Lagan tole and maximum hardness 318mg/l was recorded from tube well at Lagan tole. The source wise distribution of average hardness showed the highest figure in case of tube well water (160.63mg/l) followed by stone spouts (137 mg/l), and tap water 55.63mg/l respectively. The results showed that none of water samples exceed the WHO permissible level.

Table 5 Source wise distribution of hardness level of water samples

S.no.	Source	Hardness (mg/l)		
		Minimum	Maximum	Average
1	Stone Spouts	24.00	304.00	137.00
2	Tap Water	8.00	294.00	55.63
3	Tube Well	64.00	318.00	160.63

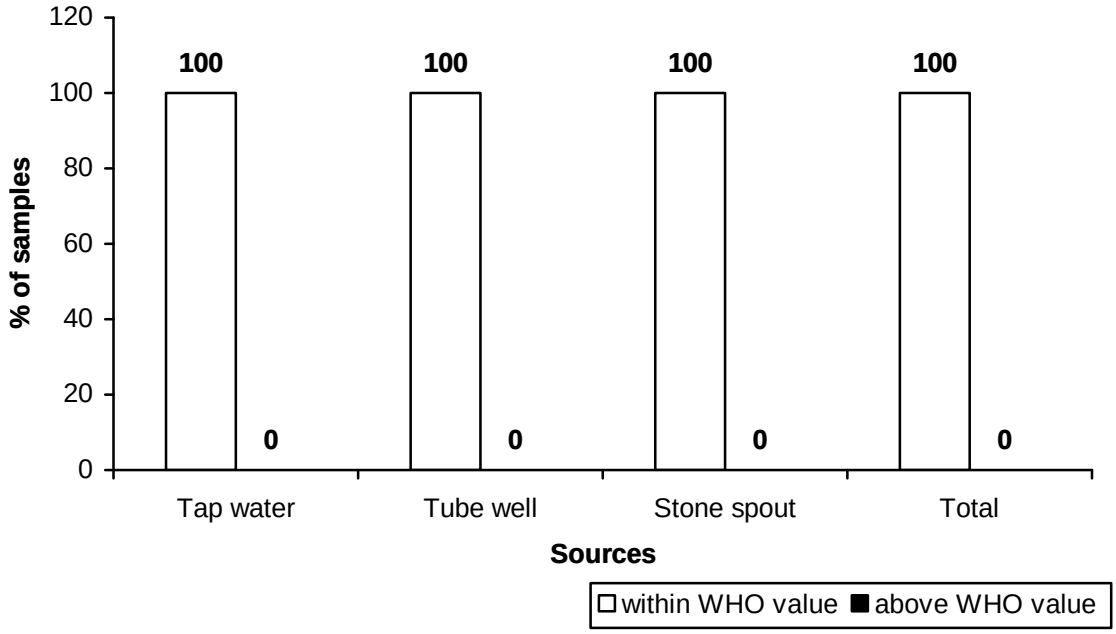


Figure 6 Comparison of hardness level of water samples with standard

Chloride

The chloride content of analysed water samples also showed a remarkable variability ranging from minimum 4.2 mg/l in many samples to maximum 230 mg/l in stone spouts water collected at Dhoka tole. None of water samples exceed the WHO permissible level out of 114 total samples.

Table 6 Source wise distribution of chloride content of water samples

S.no.	Source	Chloride (mg/l)		
		Minimum	Maximum	Average
1	Stone Spouts	5.68	230.00	53.54
2	Tap Water	4.20	29.82	10.41
3	Tube Well	12.78	218.60	76.06

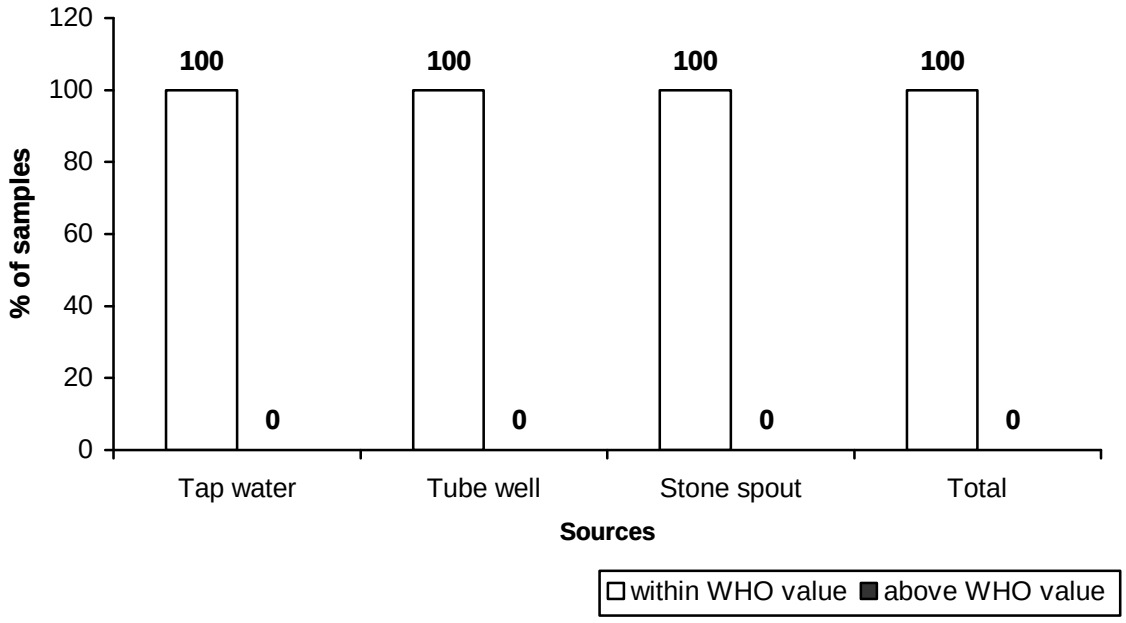


Figure 7 Comparison of chloride content of water samples with standard

Iron

The iron content of analysed water samples also showed a remarkable variability ranging from 0 mg/l in many samples of all sources to maximum 3.8 mg/l in tube well water collected at Bhedasingh. Source wise iron content showed that tube well water contained high amount of iron whereas water collected from stone spouts showed low amount of iron. The comparison test results of iron content showed most water samples 68.42% remained the limit set by WHO. However, 31.58% samples crossed the permissible guideline value as prescribed by WHO.

Table 7 Source wise distribution of iron content of water samples

S.no.	Source	Iron (mg/l)		
		Minimum	Maximum	Average
1	Stone Spouts	0.00	3.72	0.29
2	Tap Water	0.00	3.70	0.42
3	Tube Well	0.00	3.87	1.26

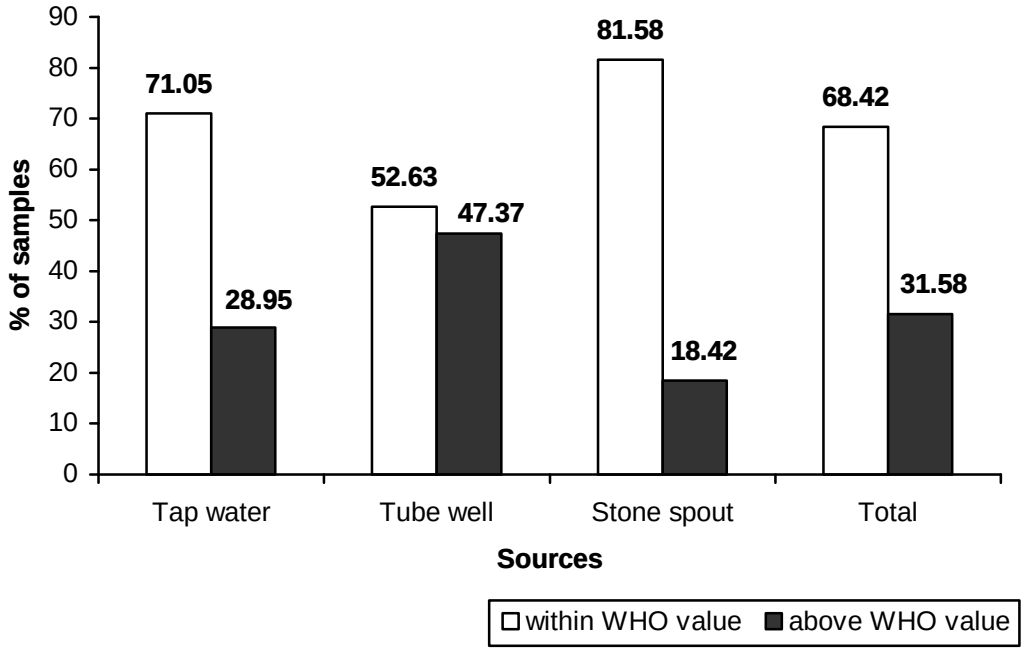


Figure 8 Comparison of iron content of water samples with standard

Ammonia

The ammonia content of analysed water samples also showed a remarkable variability ranging from minimum 0 mg/l in many samples of all sources to maximum >3 mg/l in many samples. Source wise ammonia content revealed that tube well waters were highly contaminated with ammonia (31.58%) whereas waters collected from taps were least i.e (15.79%)

Table 8 Source wise distribution of ammonia content of water samples

S.no.	Source	Ammonia (mg/l)		
		Minimum	Maximum	Average
1	Stone Spouts	0.00	>3	0.71
2	Tap Water	0.00	>3	0.70
3	Tube Well	0.00	>3	1.46

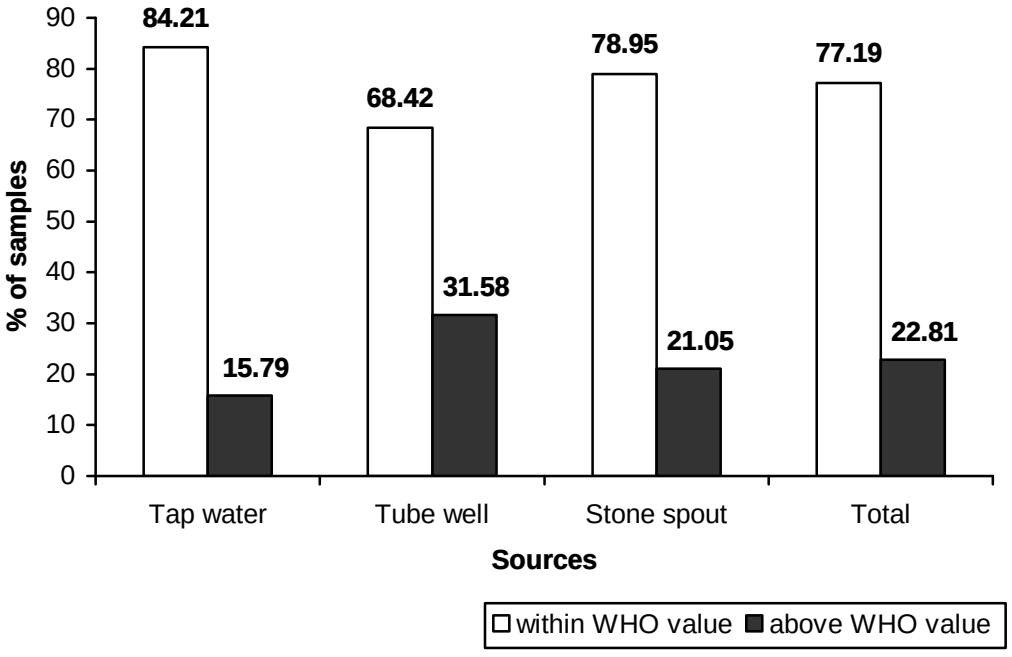


Figure 9 Comparison of ammonia content of water samples with standard

Nitrate

NItrate was another chemical parameter in which notable variability was experienced. The nitrate content values ranged from 0 mg/l in many samples to >50 mg/l in tube well water at Tahachal. A comparison with permissible level showed that majority of water samples (99.12%) remained within limit set by WHO with only one samples crossing the limit i.e. tube well water collected from Tahachal.

Table 9 Source wise distribution of nitrate content of water samples

S.no.	Source	Nitrate (mg/l)		
		Minimum	Maximum	Average
1	Stone Spouts	0.00	30.00	9.61
2	Tap Water	0.00	30.00	3.37
3	Tube Well	0.00	>50.00	8.34

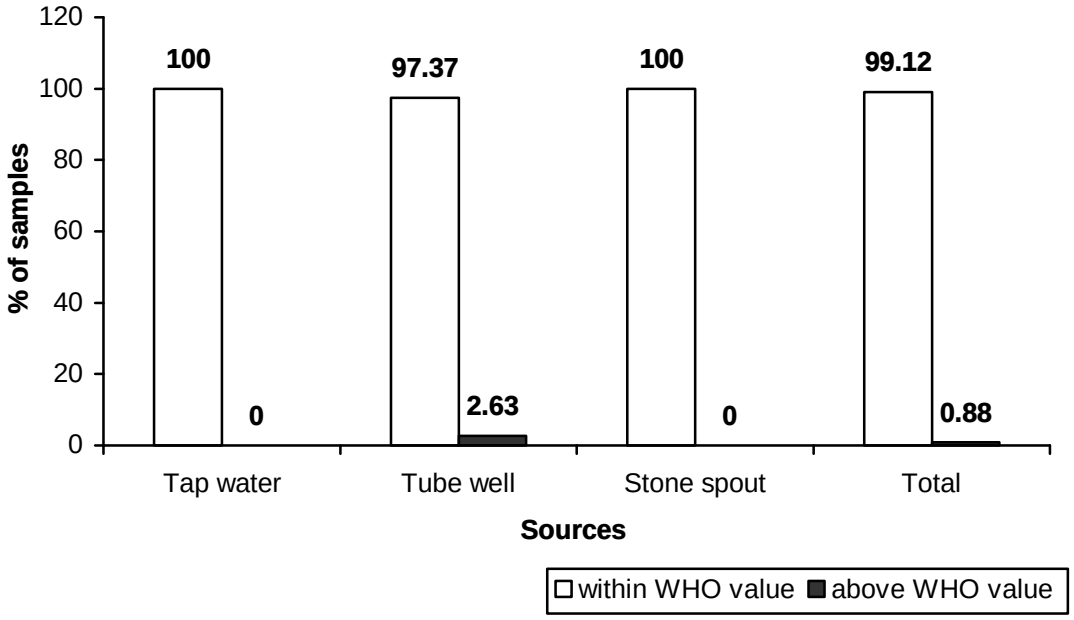


Figure 10 Comparison of nitrate content of water samples with standard

Arsenic

Seven samples were recorded to be contaminated with arsenic during study period. The maximum arsenic content value 0.1mg/l was obtained from tube well of Kuleswor. Comparing sources, 2 tap water samples and 5 tube well samples were found to cross the WHO limit whereas none of the sample from stone spouts were found contaminated. Out of 114 samples, 93.86 % were within standard and 6.14% were above standard value.

Table 10 Source wise distribution of arsenic content of water samples

S.no.	Source	Arsenic (mg/l)		
		Minimum	Maximum	Average
1	Stone Spouts	0.0	0.010	0.002
2	Tap Water	0.0	0.050	0.003
3	Tube Well	0.0	0.100	0.004

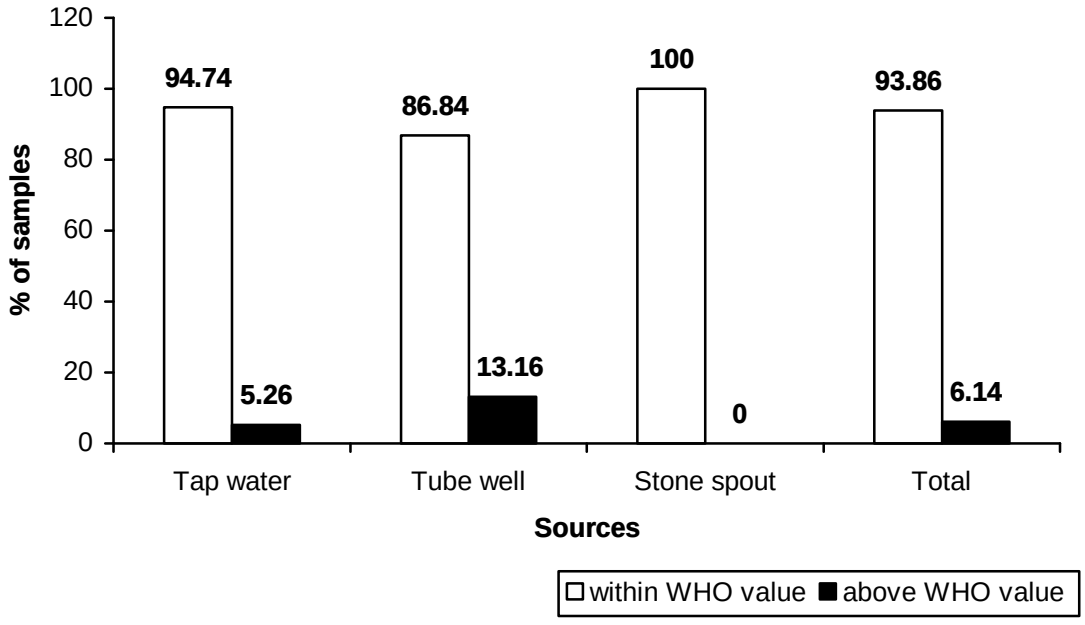


Figure 11 Comparison of arsenic content of water samples with standard

5.2 Bacteriological Analysis of Water

Total of one hundred fourteen samples were analysed for bacteriological study during study period from different sources of Kathmandu metropolitan areas. The membrane filter method was applied for total enumeration of coliform bacteria.

Coliform count

The microbiological analysis of water revealed the presence of total coliforms in 90.35 % (103) of total samples i.e the majority of water samples taken were found positive for total coliforms. Only 26.32% tap water and 2.63% tube well water were *total coliform count* negative.

Source wise distribution of coliform count clearly showed that the water samples from (26.32%) tap water, (2.63%) tube well water and (0%) stone spout were within the WHO guideline value. Like wise tube well (97.37 %), tap (73.68%) and stone spout (100 %) crossed the WHO guideline value.

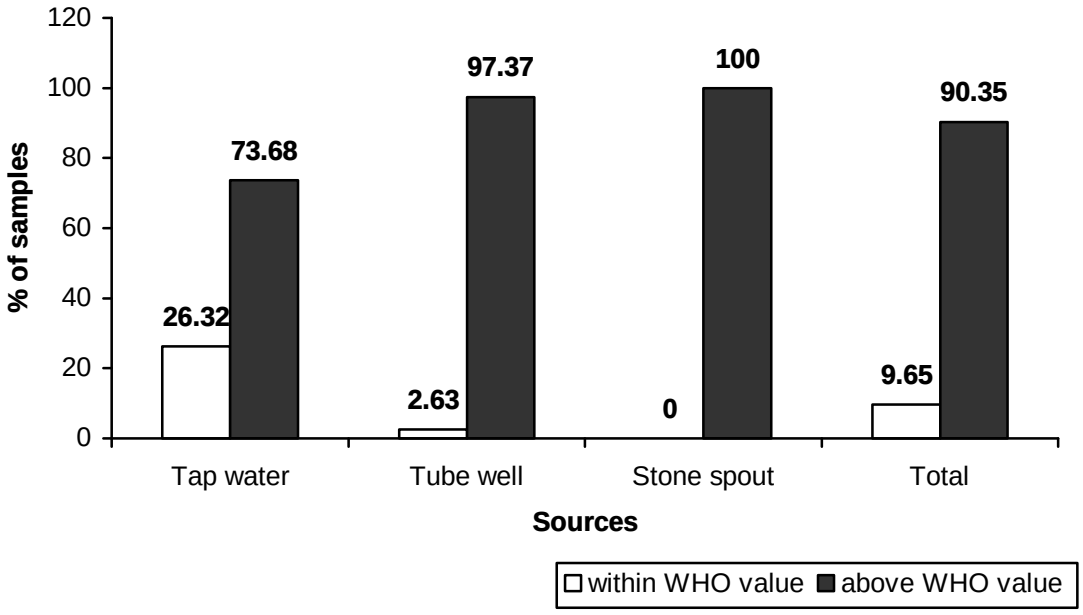


Figure 12 Source wise quality of coliform count of water samples

Isolation and Identification of isolated bacteria

Ten different kinds of enteric bacteria were isolated from 114 drinking water samples collected from different sources of different places in Kathmandu. The isolated organisms were subjected to various biochemical tests for their identification. The organisms identified include *Escherichia coli, Klebsiella* spp*, Citrobacter* spp*, Enterobacter* spp*, Salmonella typhi, Salmonella paratyphi, Shigella* spp *Proteus vulgaris, Proteus mirabilis and Pseudomonas* spp.

Among the isolates, *Citrobacter* spp (26.22%) was found to be maximum followed by *Escherichia coli* (25%), *Enterobacter* spp (20.73%), *Shigella* spp (8.54%), *Proteus vulgaris*(7.93%) *Pseudomonas aeruginosa* (3.66%), *Salmonella paratyphi* (3.05%) *Klebsiella* spp (2.44%), *Proteus mirabilis* (1.83%) and *Salmonella typhi* (0.61%). During study period, *Vibrio cholerae* was not detected from any sample. Percentage of different enterobacteria isolated was presented in Table 12.

Table 11 Source wise distribution of enteric bacterial isolates of water samples

S.N	**Source**	*E. coli*	*Klebsiella* spp.	*Citrobacter* spp.	*Enterobacter* spp.	*Salmonella typhii*	*Salmonella paratyphi*	*Shigella* spp.	*Proteus vulgaris*	*Proteus mirabilis*	*Pseudomonas* spp.	*Vibrio cholerae*	**Total**
1	Stone spout	9	2	14	9	0	3	7	5	3	2	0	54
2	Tap water	15	0	13	8	1	2	2	4	0	1	0	46
3	Tube well	17	2	16	17	0	0	5	4	0	3	0	64
	Total	41	4	43	34	1	5	14	13	3	6	0	164

Correlation among different parameters for water samples:

The water analysis data showed significant positive correlation between conductivity and hardness values for all water source types. Similarly, conductivity correlates significantly with chloride for tube well and stone spouts. The findings also showed a significant positive correlation of conductivity with nitrate, arsenic ammonia and iron in tap water and

conductivity with nitrate and arsenic in stone spouts. The turbidity and iron values for tap water exhibited significant positive correlation.

Table 12 Percentage of different enterobacteria isolated from water samples

S.N	Isolated enteric bacteria from water samples	Number	Percentage
1	*E. coli*	41	25.00
2	*Klebsiella* spp.	4	2.44
3	*Citrobacter* spp.	43	26.22
4	*Enterobacter* spp.	34	20.73
5	*Salmonella typhi*	1	0.61
6	*Salmonella paratyphi*	5	3.05
7	*Shigella* spp.	14	8.54
8	*Proteus vulgaris*	13	7.93
9	*Proteus mirabilis*	3	1.83
10	*Pseudomonas* spp.	6	3.66
11	*Vibrio cholerae*	0	0.00
	Total	164	100.00

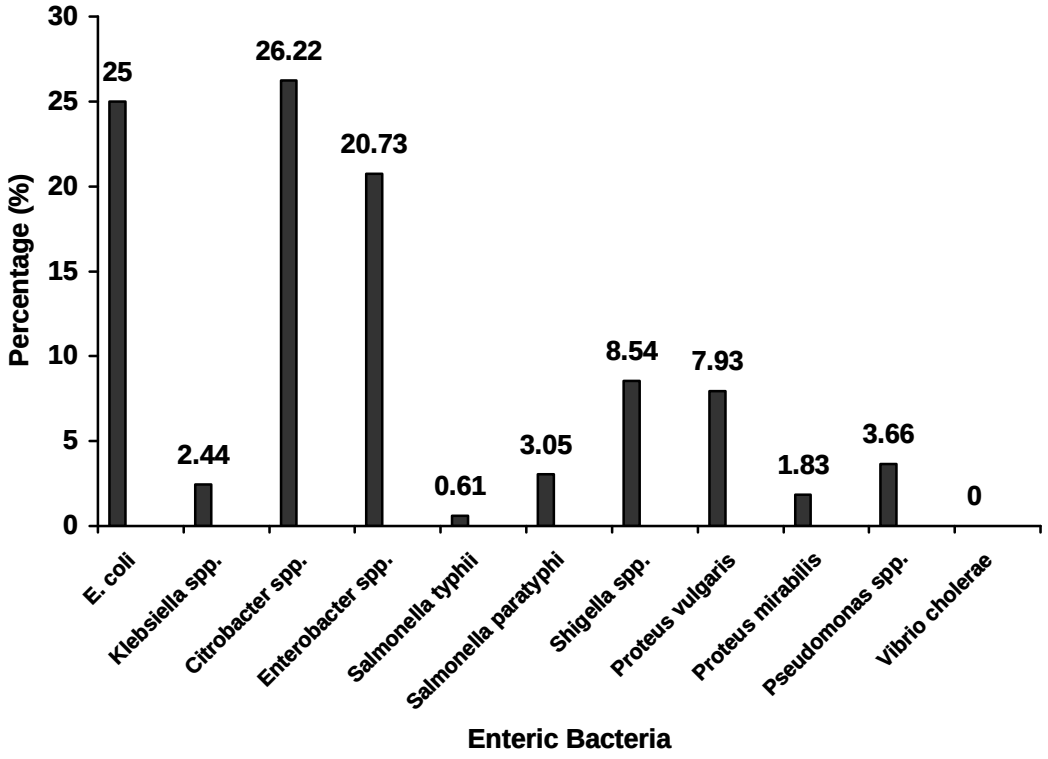


Figure 13 Percentage of different enterobacteria isolated from water samples

Table 1 Correlation among different parameters for water samples from tube well

Test Parameters	Temperature	pH	Conductivity	Turbidity	Hardness	Chloride	Iron	Ammonia	Nitrate	Total Coliform Count
Temperature	1	0.06	0.12	-0.63	-0.21	0.01	-0.17	-0.04	0.16	0.36
pH		1	0.08	0.04	0.40	-0.17	-0.25	0.23	0.35	0.17
Conductivity			1	-0.17	0.50*	0.83*	-0.24	0.45	0.25	0.16
Turbidity				1	0.11	-0.02	0.31	-0.08	-0.14	-0.27
Hardness					1	0.33	-0.35	0.48	0.24	-0.03
Chloride						1	-0.11	0.30	0.11	-0.02
Iron							1	0.01	-0.42	-0.12
Ammonia								1	0.16	0.07
Nitrate									1	0.28
Total Coliform Count										1

* Correlation is significant.

Table 2 Correlation among different parameters for water samples from tap

Test Parameters	Temperature	pH	Conductivity	Turbidity	Hardness	Chloride	Iron	Ammonia	Nitrate	Total Coliform Count
Temperature	1	0.03	0.31	0.13	0.27	-0.17	0.09	0.14	0.13	-0.03
pH		1	0.22	0.16	0.26	-0.09	0.33	0.17	0.02	-0.06
Conductivity			1	0.76*	0.92*	0.06	0.73*	0.75*	0.14	0.20
Turbidity				1	0.78*	0.14	0.74*	0.67*	-0.14	0.35
Hardness					1	0.09	0.70*	0.72*	0.13	0.23
Chloride						1	0.00	-0.08	-0.16	-0.10
Iron							1	0.64*	-0.09	0.32
Ammonia								1	0.31	0.45
Nitrate									1	0.27
Total Coliform Count										1

* Correlation is significant.

Table 3 Correlation among different parameters for water samples from stone spout

Test Parameters	Temperature	pH	Conductivity	Turbidity	Hardness	Chloride	Iron	Ammonia	Nitrate	Total Coliform Count
Temperature	1	-0.08	0.07	-0.20	0.05	0.04	0.11	-0.30	-0.07	-0.07
pH		1	-0.15	0.20	0.05	-0.11	-0.08	-0.23	-0.10	0.20
Conductivity			1	-0.19	0.77*	0.86*	-0.05	0.33	0.52*	0.40
Turbidity				1	-0.28	-0.05	0.36	-0.10	-0.01	-0.18
Hardness					1	0.70*	-0.10	0.17	0.50*	0.42
Chloride						1	0.01	0.18	0.31	0.43
Iron							1	0.00	-0.12	-0.13
Ammonia								1	0.19	0.20
Nitrate									1	0.14
Total Coliform Count										1

* Correlation is significant.

5.3 Antibacterial Properties of Medicinal Plants

Percentage Yield of Ethanol Crude Extracts of Medicinal Plants

The percentage yields of the ethanol crude extract of medicinal plants as obtained by soxhlet extraction process are shown in the Table 16. The amount of crude extracts varied among the medicinal plants. *Punica granatum* gave the highest yield (25.30%) and lowest yield was obtained from *Anethum sowa* (7.59%).

Table 16: Percentage yields of ethanol crude extracts of medicinal plants

S.no.	Name of medicinal plants	Percentage Yields
1	*Anethum sowa*	7.59
2	*Aegle marmelos*	14.14
3	*Punica granatum*	25.30
4	*Woodfordia fruticosa*	24.8
5	*Mimosa pudica*	24.2
6	*Psidium guajava*	16.87
7	*Syzygium cumini*	21.40
8	*Acorus calamus*	15.50

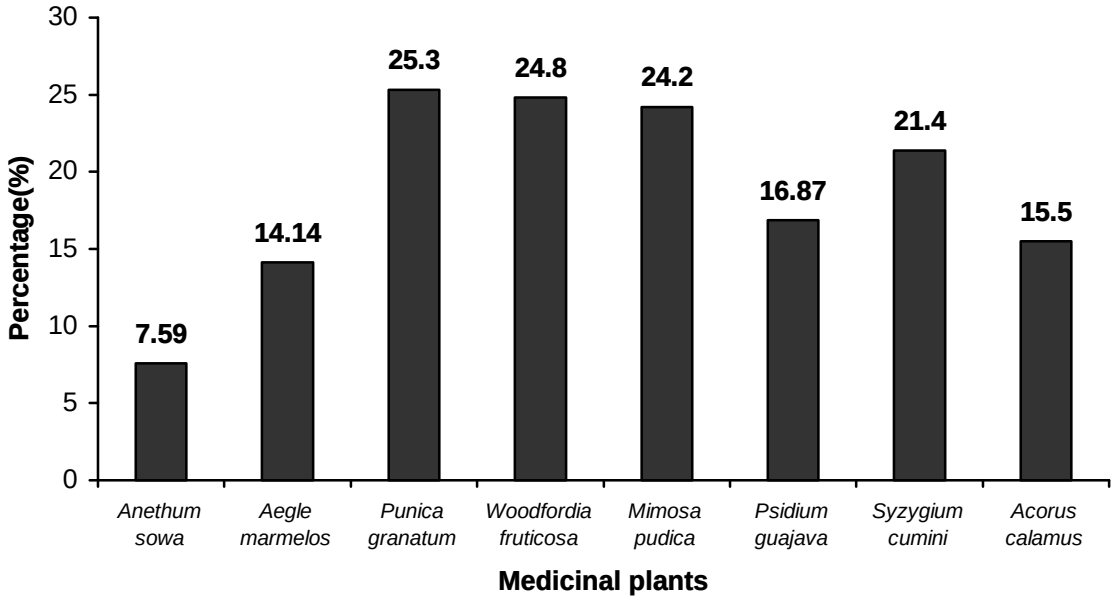


Figure 14 Percentage yields of ethanol crude extracts of medicinal plants

Screening of medicinal plants for antibacterial activity

The data pertaining to the antimicrobial potential of the plant extracts are presented in the Table 17. It is revealed from the table that out of eight medicinal plants selected, four plants *Punica granatum* (Anar), *Woodfordia fruticosa* (Dhanyero), *Psidium guajava* (Amba) *and Syzygium cumini* (Jamun) were found to be effective against all the enteric bacteria isolated.

Table 17: Antibacterial properties of ethanolic extracts of different medicinal plants against tested bacteria

S. N	Medicinal Plants	*E. coli*	*Klebsiella* spp.	*Citrobacter* spp.	*Enterobacter* spp.	*Salmonella typhii*	*Salmonella paratyphi*	*Shigella* spp.	*Proteus vulgaris*	*Proteus mirabilis*	*Pseudomonas* spp.
1	*Anethum sowa*	-	-	-	-	-	-	-	-	-	-
2	*Aegle marmelos*	-	-	-	-	-	-	-	-	-	-
3	*Punica granatum*	+	+	+	+	+	+	+	+	+	+
4	*Woodfordia fruticosa*	+	+	+	+	+	+	+	+	+	+
5	*Mimosa pudica*	-	-	-	-	-	-	-	-	-	-
6	*Psidium guajava*	+	+	+	+	+	+	+	+	+	+
7	*Syzygium cumini*	+	+	+	+	+	+	+	+	+	+
8	*Acorus calamus*	-	-	-	-	-	-	-	-	-	-

Notes: -Positive (+) sign indicates the ethanolic extracts of the particular medicinal plants inhibited the growth of microorganisms & thus produce zone of inhibition. Absence of zone of inhibition was denoted as negative (-) sign.

Evaluation of antibacterial activity of medicinal plants.

The antibacterial activity of medicinal plants were evaluated by two ways viz. measuring zone of inhibition and quantitative determination of plant extract for minimum bactericidal concentration (MBC). The diameter of zone of inhibition (ZOI) produced by plant extract on particular bacteria was measured for the estimation of potency of plant extract. Similarly, the minimum amount of extract usually expressed in terms of microgram or milligram per milliliter of the bacterial broth solution required to inhibit the growth as well as to kill the bacteria altogether clearly expresses the better effectiveness of the extract against the corresponding bacteria. The mean diameter of zone of inhibition and minimum bactericidal

concentration of ethanol extract of different medicinal plants which showed significant zone of inhibition (>8mm) during qualitative screening process (as indicated by +sign in table 17) are shown in tables below.

Table 18: Mean diameter of zone of inhibition (ZOI) and minimum bactericidal concentration (MBC) given by *Punica granatum* against enteric bacteria

S.N	Test organism	Antimicrobial activity	
		ZOI (mm)	MBC (mg/ml)
1	*E. coli*	21	50
2	*Klebsiella* spp.	27	6.25
3	*Citrobacter* spp.	25	25
4	*Enterobacter* spp.	23	50
5	*Salmonella typhii*	19	3.12
6	*Salmonella paratyphi*	25	1.56
7	*Shigella* spp.	23	50
8	*Proteus vulgaris*	16	6.25
9	*Proteus mirabilis*	30	12.5
10	*Pseudomonas* spp.	11	50

Note: During determination of zone of inhibition the well diameter 6mm being included. The height of the well was 4mm and the concentration of extract loaded (50µl) in each well was 100mg/ml.

Punica granatum was effective against all the enteric bacteria isolates so may be considered to have broad antibacterial activity. The activity of *Punica granatum* against *Escherichia coli*, *Enterobacter* spp, *Shigella* spp and *Pseudomonas* spp was weak as evidenced by MBC value of 50mg/ml. *Salmonella paratyphi* seemed most susceptible, having MBC value of 1.56mg/l.

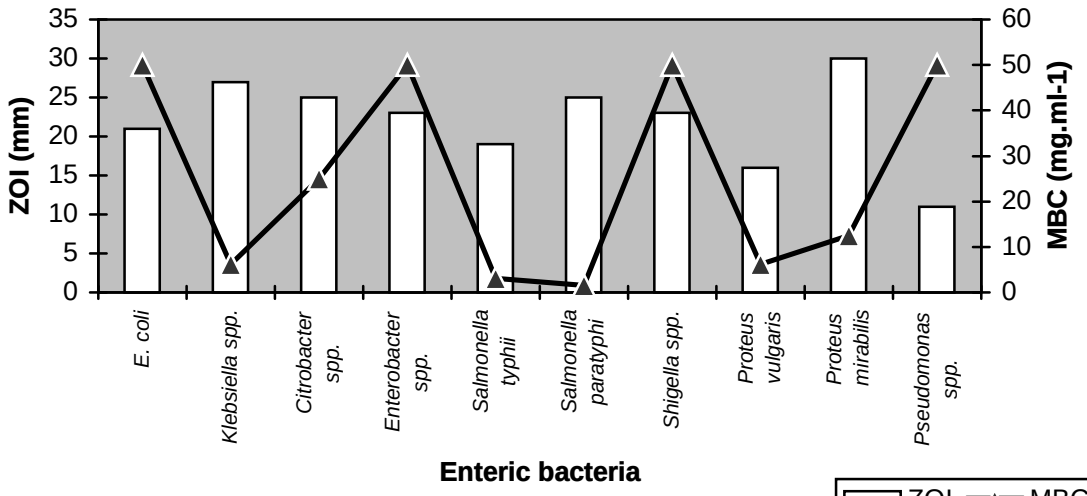


Figure 15 Zone of Inhibition (ZOI) and Minimum Bactericidal Concentration (MBC) given by *Punica granatum* against tested bacteria.

Table 19 Mean diameter of zone of inhibition (ZOI) and minimum bactericidal concentration (MBC) given by *Woodfordia fruticosa* against tested bacteria

S.N	Test organism	Antimicrobial activity	
		ZOI (mm)	MBC (mg/ml)
1	*E. coli*	16	50
2	*Klebsiella* spp.	20	50
3	*Citrobacter* spp.	20	50
4	*Enterobacter* spp.	18.5	50
5	*Salmonella typhii*	26.5	1.56
6	*Salmonella paratyphi*	15	25
7	*Shigella* spp.	15	25
8	*Proteus vulgaris*	22	1.56
9	*Proteus mirabilis*	23	25
10	*Pseudomonas* spp.	15	50

Woodfordia fruticosa may be considered to have broad antibacterial activity, inhibiting growth of 10 out of 10 test organisms. The activity of *Woodfordia fruticosa* against *E. coli* , *Klebsiella* spp., *Citrobacter* spp, *Enterobacter* spp and *Pseudomonas* spp was weak though as evidenced by MBC equals to 50mg/ml. *Salmonella typhi* seemed most susceptible, having highest ZOI as well as MBC value of 1.56mg/ml.

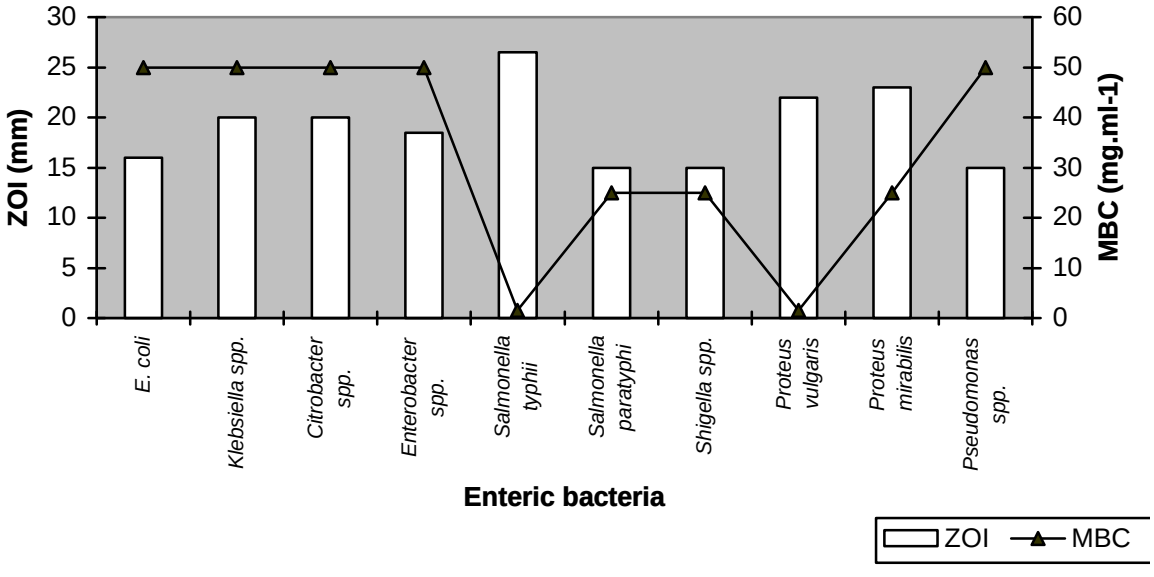


Figure 16 Zone of Inhibition (ZOI) and Minimum Bactericidal Concentration (MBC) given by *Woodfordia fruticosa* against tested bacteria

Table 20 Mean diameter of zone of inhibition (ZOI) and minimum bactericidal concentration (MBC) given by *Psidium guajava* against tested bacteria

S.N	Test organism	Antimicrobial activity	
		ZOI (mm)	MBC (mg/ml)
1	*E. coli*	16	50
2	*Klebsiella* spp.	25.5	50
3	*Citrobacter* spp.	16	50
4	*Enterobacter* spp.	19.5	50
5	*Salmonella typhii*	13	6.25
6	*Salmonella paratyphi*	20	3.12
7	*Shigella* spp.	14	50
8	*Proteus vulgaris*	15	25
9	*Proteus mirabilis*	24	12.5
10	*Pseudomonas* spp.	10	50

Psidium guajava exhibited inhibitory effect against all 10 enteric bacteria isolates. It showed highest activity against *Salmonella paratyphi* and lowest against *E. coli, Klebsiella* spp, *Citrobacter* spp, *Enterobacter* spp, *Shigella* spp and *Pseudomonas* spp. Thus, *Salmonella paratyphi* seemed most susceptible, having MBC value of 3.12mg/ml.

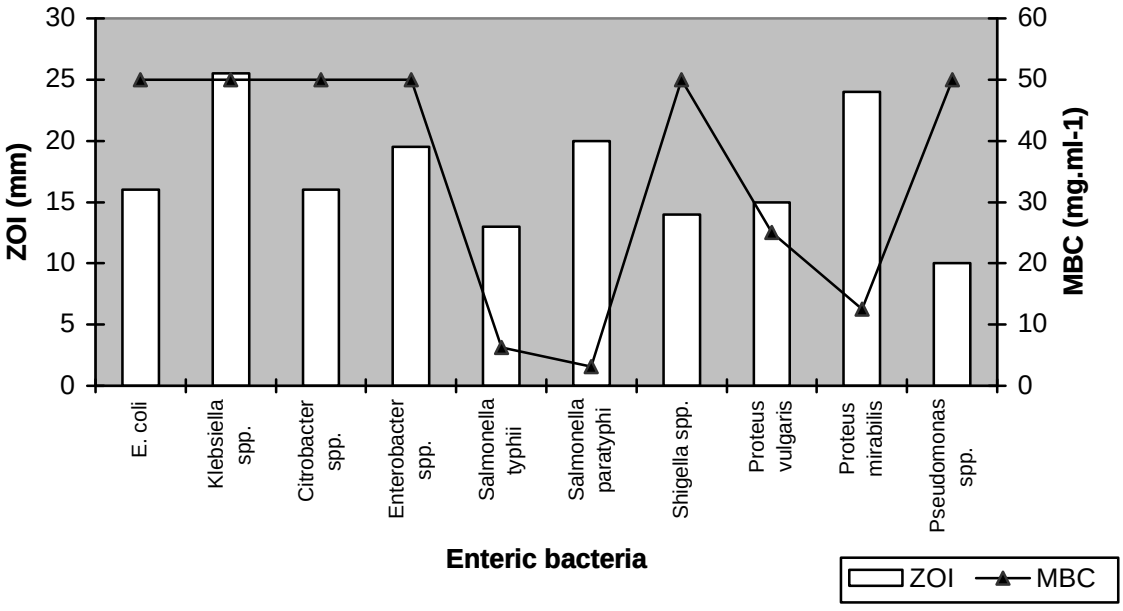


Figure 17 Zone of Inhibition (ZOI) and Minimum Bactericidal Concentration (MBC) given by *Psidium guajava* against tested bacteria

Table 21 Mean diameter of zone of inhibition (ZOI) and minimum bactericidal concentration (MBC) given by *Syzygium cumini* against tested bacteria

S.N	Test organism	Antimicrobial activity	
		ZOI (mm)	MBC (mg/ml)
1	*E. coli*	21	50
2	*Klebsiella* spp.	25	50
3	*Citrobacter* spp.	20	25
4	*Enterobacter* spp.	22.5	50
5	*Salmonella typhii*	10.5	3.12
6	*Salmonella paratyphi*	20	0.39
7	*Shigella* spp.	19.5	50
8	*Proteus vulgaris*	18	1.56
9	*Proteus mirabilis*	31	12.5
10	*Pseudomonas* spp.	11.5	25

Syzygium cumini inhibited the growth of all 10 isolates thus may be considered to have broad antibacterial activity. The activity of *Syzygium cumini* against *E coli*, *Klebsiella spp*, *Enterobacter* spp and *Shigella* spp was weak though as evidenced by MBC 50mg/ml. *Salmonella paratyphi* seemed most susceptible, having MBC value of 0.39mg/ml.

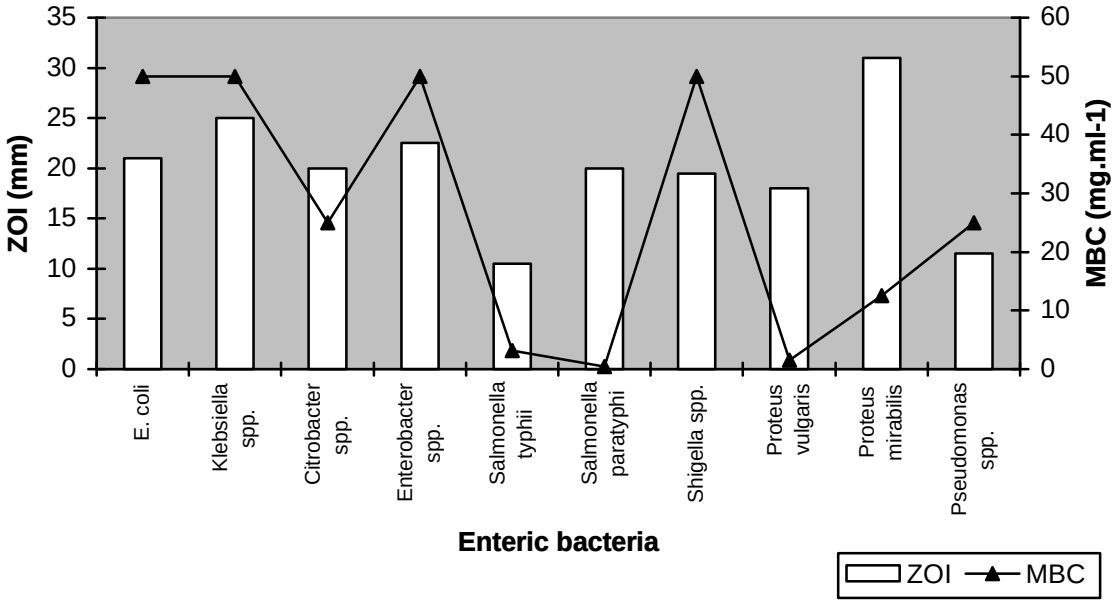
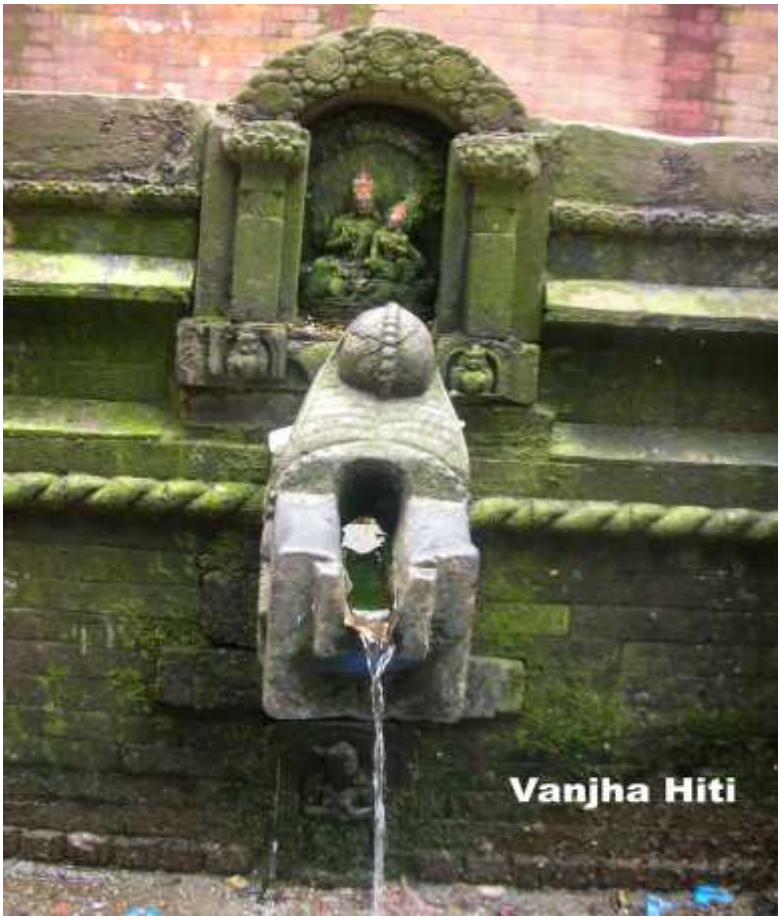


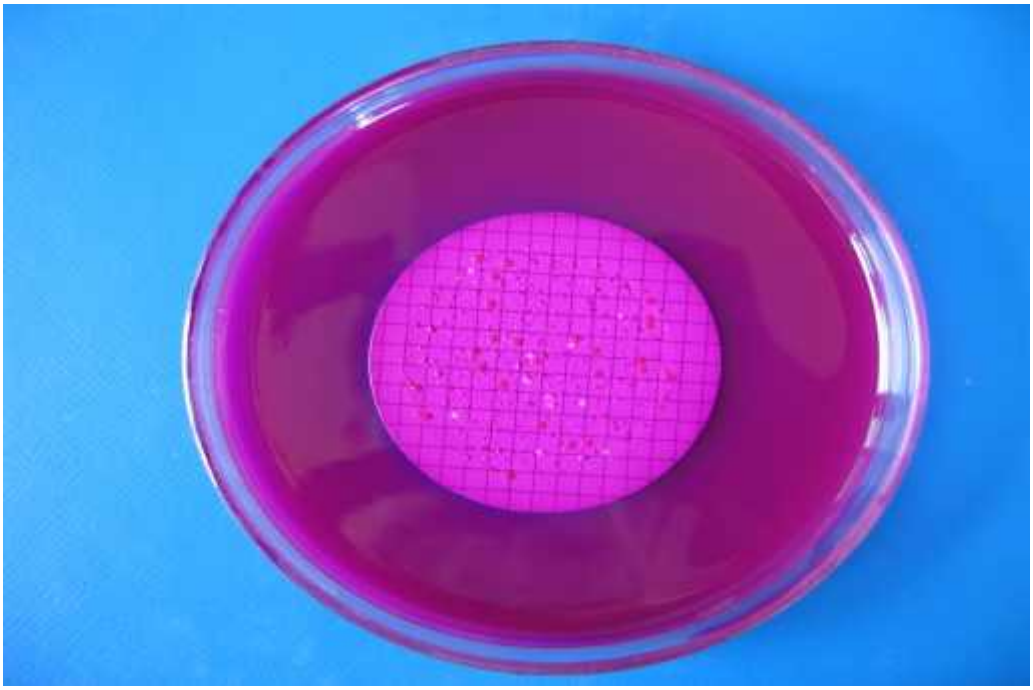
Figure 18 Zone of Inhibition (ZOI) and Minimum Bactericidal Concentration (MBC) given by *Syzygium cumini* against tested bacteria



Photograph 1: Stone spout (Vanjha hiti) at Maru

Photograph 2: Soxhlet extractor showing ethanol extraction of medicinal plants

Photograph 3: Arsenic determination by Quantofix test kit

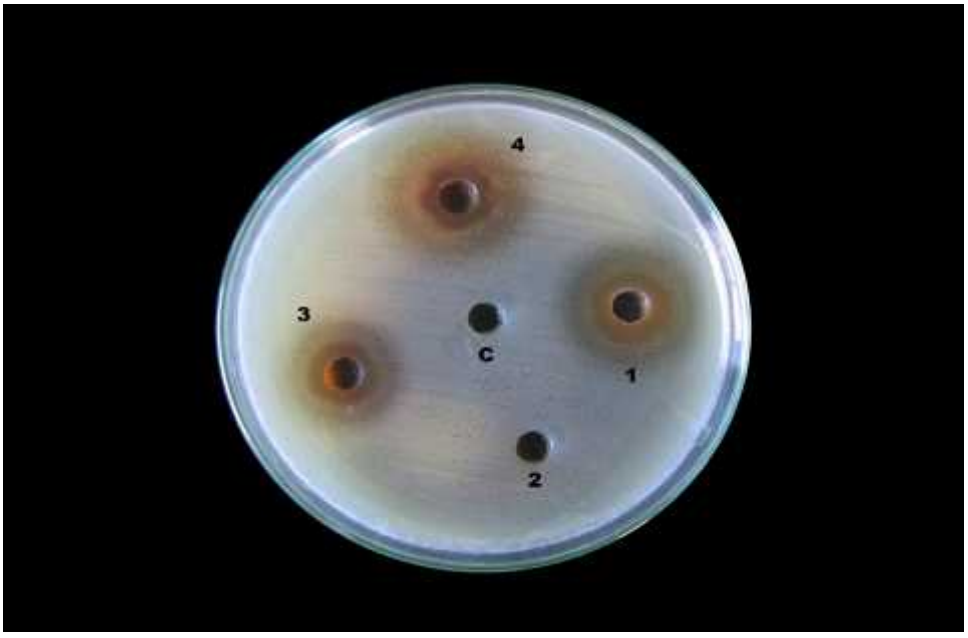


Photograph 4: Colonies of Total Coliforms on M-Endo agar

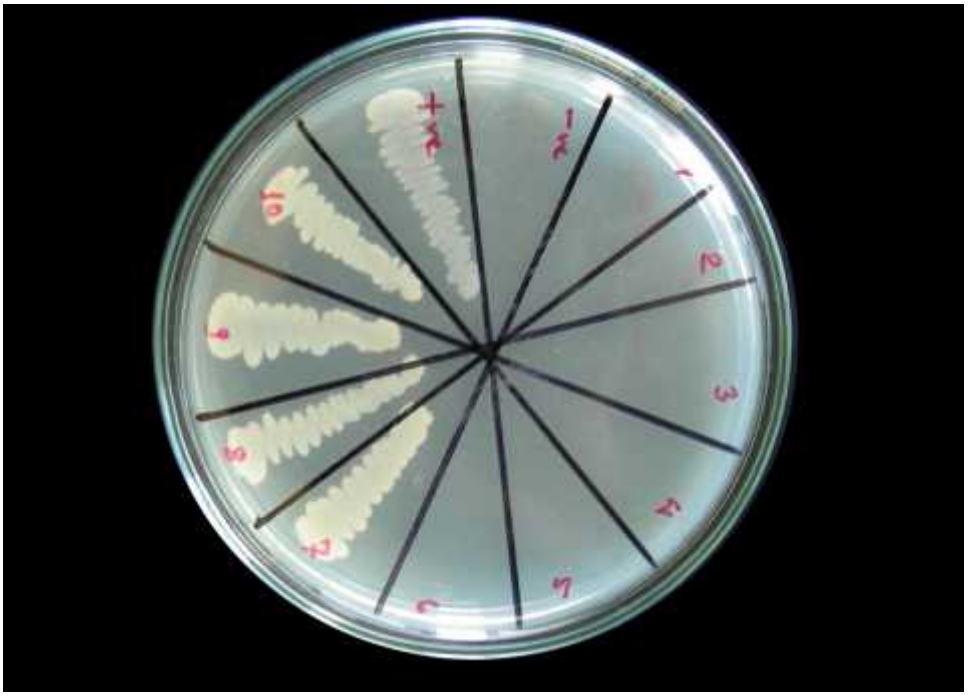
Photograph 5: Tubes showing results of biochemical tests for *E. coli*

Photograph 6: Different Medicinal Plants

Photograph 7: Zone of inhibition produced by medicinal plant extracts against *Pseudomonas aeruginosa.*1. *Woodfordia fruticosa* (+), 2.*Anethum sowa* (-) 3.*Mimosa pudica* (-) 4. *Aegle marmelos* (-), C-control



Photograph 8: Zone of inhibition produced by medicinal plant extracts against *Proteus vulgaris.* 1. *Punica granatum* (+), 2. *Acorus calamus* (-), 3. *Psidium guajava* (+), 4. *Syzygium cumini* (+), C-control.



Photograph 9: Minimum bactericidal concentration (MBC) value (1.56mg/l) shown by *Punica granatum* against *Salmonella paratyphi*

CHAPTER-VI

6 DISCUSSION AND CONCLUSION

6.1 Discussion

In the present study 114 different drinking water samples were analysed to assess the existing status of physical, chemical and bacteriological quality of drinking water sources of KMC. In addition, evaluation of antibacterial properties of some medicinal plants against the enteric bacteria isolates was also carried out.

6.1.1 Physico-chemical parameters of water

These samples were analysed for physicochemical parameters such as temperature, pH, conductivity, turbidity, hardness, chloride, iron content, ammonia, nitrate and arsenic. No standards or guideline values regarding the temperature have been set. However, the test results of temperature showed the remarkable variation ranging from the minimum temperature (18^0C) in the month of February in tube well water collected from Bhotebahal to the maximum temperature (30.2^0C) was recorded in the month of June from Tulsi dhara, Dallu area. This result revealed that water bodies undergo temperature variation along with normal climatic fluctuations and seasonal variation. High water temperature enhances the growth of microorganisms and may increase taste, odour, colour and corrosion problem.

The test results of pH values showed 85.09% of water samples were within the permissible limit, whereas 14.91% showed their pH values below the permissible and none of samples crossed the upper limit of guideline value. Maharjan (1998) had shown similar result with 88.6% within the permissible limit and 11.4% below the permissible. A study conducted jointly by JICA/ENPHO (2005) showed that out of total 134 samples tested in pre-monsoon, the pH value for one-third of the water samples (34.3%) was found to be below WHO guideline value, 65.7% samples within WHO guideline value and none of the sample exceeded the maximum WHO permissible limit. In drinking water, pH less than 7.0 may

cause corrosion of metal pipes in the distribution system and higher than 8.0 adversely affect the disinfection process. Chlorination may be markedly less effective in increasing pH values. The electrical conductivity measurement values ranged from 21 µs/cm in tap water sample to 1130 µs/cm in tube well water at Lagan tole. Comparision with permissible level showed that majority of water samples (75.44%) remained within limit set by WHO. However, 24.56% samples crossed the limit. Conductivity is the ability of water to conduct electric current and is directly related to the amount of salt dissolved in it. It doesn't have a direct health effect. However, high conductivity most of the time is due to the pollution. Tube wells had comparatively higher average conductivity than other two sources which may be due to pollution in dug wells and natural property in deep wells.

Turbidity in water is caused by the presence of suspended and colloidal matter. Usually, water with high turbidity has offensive appearance, color, taste and odor. The main problem associated with turbidity is the microbiological quality since its presence can interfere with the detection of bacteria and viruses. Disinfection of water becomes less effective because the microorganisms are protected by the particles that cause turbidity. The WHO guideline value for turbidity is 5 NTU but is based on consumer complaints, not the health. The WHO limit for turbidity has been violated by 26.31% of the samples. The source wise turbidity measurement showed that tap water and tube well water were highly turbid, whereas water collected from stone spouts were least turbid.

Hardness of water is predominantly due to dissolved calcium and magnesium. Studies carried out so far do not reveal direct and conclusive health effect due to hardness in water. Increase in hardness has been found to be beneficial to human health in some studies. The WHO guideline value for hardness is 500 mg/L as CaCO3. The test result of hardness showed almost all water samples within the WHO permissible limit. Similar results were obtained by Pradhananga *et al* (1993), Ghimire (1996), Thapa (1997) and Prasai (2004).

Chloride can be an indicator of pollution. Usually the high concentrations of chloride in combination with nitrate or ammonium show that the water is contaminated by the domestic sources. The test result of chloride showed all water samples within the WHO permissible

limit. Thapa (1997) and JICA/ENPHO (2005) also found that chloride content for all water samples were within the WHO guideline value.

Iron can be present in water naturally and due to pollution also. Iron in higher concentration can be aesthetically unpleasant and can stain clothes, hair and nails. WHO guideline value of iron is 0.3 mg/l. Some of water samples 31.58% exceeded the WHO guideline value and 68.42% water samples were within the limit set by WHO. Tube wells and stone spouts water had higher concentration iron than tap water. Surface water generally contains little amounts of iron while the ground water may contain very high amount.

Ammonia content in water may be harmful to health since it can be converted to nitrate. Presence of ammonia does not always mean that it is due to domestic pollution. Many deep wells also contain a lot of ammonia and it is believed to be because of underlying geochemistry. In the present study the WHO limit of 1.5mg/l was violated by 22.8% of the samples. High concentrations of ammonia have been found in tube wells and stone spouts when compared to that in tap water. High ammonia content in deep well can be due to the underlying intercalated layers of peat and lignite. The low ammonia content in some cases may be due to the microbial conversion of ammonia to nitrate.

Nitrate can be added to water from agriculture applications, domestic sewer and industrial effluents. Organic nitrogen in the waste water is first converted to ammonium which is then converted to nitrite and nitrate by microbial action. High concentration of nitrate could be harmful to human health, especially, to infants and young children. In babies, it can cause a disease called “methemoglobinemia”. The disease is caused by the conversion of nitrate to nitrite which makes haemoglobin unable to carry oxygen. Tap water had very low nitrate concentration and none of the tap water along with stone spouts violated the WHO limit. Only one tube well sample from Tahachal cross WHO limit of 50mg/L.

Arsenic poisoning in the ground water of Terai in Nepal is becoming a new challenge for the nation’s water supply sector. Presence of arsenic in groundwater of Nepal’s Terai districts was known for the first time in 1999 (Sharma, 1999). In the present study, arsenic contamination in groundwater of Kathmandu Valley has been observed, the highest

concentration of arsenic (0.1mg/l.) was obtained from tube well at Kuleswor and a study conducted jointly by JICA/ENPHO (2005) also showed the highest arsenic concentration of 265 ppb in Kuleswor. The study also revealed that majority of wells exceeding WHO lie in Kalimati areas. Out of 114 samples, 93.86 % were within standard and 6.14% were above standard value. Thus water from some areas of Kathmandu was found to be contaminated with arsenic. Arsenic is introduced into water through the dissolution of mineral and ores from industrial effluent and via atmospheric deposition.

6.1.2 Microbial quality of water

Health is determined by many factors, including income, environmental conditions like access to adequate sanitation and safe drinking water suppliers, behaviour change and availability of health services. More than half of the world's population lives in villages in rural areas and most of those without access to safe drinking water supply or basic sanitation are rural dwellers (Howard *et al,* 2002). The majority of diseases in developing countries are infectious in nature caused by bacteria, viruses and other microbes, which are shed in human faeces and pollute water supplies which people use for drinking and washing purposes. The various studies related to drinking water quality also showed that the water sources from the rural areas of developed countries were found to be contaminated with coliforms. The fact was clarified in the studies done by Zhang *et al* (1997), Nola *et al* (1998), Anwar *et al* (1999), India assessment (2002), Jabu (2005) and Venus (2005).

The water samples analysed from the public taps and treatment plants of the distribution system at various parts of the country, including Kathmandu has revealed that the water supply system in Nepal is very poor. Almost all studies carried out to date suggest that the water quality is deteriorating with time in all types of sources. Problems associated with the lack of clean drinking water and polluted water bodies are common in Kathmandu. In the present study, the microbiological analysis of water revealed the presence of total coliforms in 90.35 % of total samples i.e. the majority of water samples taken were found positive for total coliforms. Only 26.32% tap water and 2.63% tube well water were total coliform count negative. Source wise distribution of coliform count clearly showed that tube well (97.37 %), tap (73.68%) and stone spout (100 %) crossed the WHO guideline value. Many previous

studies indicate that the water supply in Kathmandu was far from satisfactory in terms of bacterial contamination.

Karmacharya *et al* (1991/1992)in the Kathmandu showed that out of 172 samples from 5 treatment plants and public taps, 50% of water samples of city water supply were found contaminated with coliform. ENPHO/DISVI (1990) showed that samples from all the spouts were faecally contaminated. ENPHO/DISVI (1992) found 50% samples from public taps were contaminated with faecal coliform. Bottino *et al* (1999) carried out the study regarding the water quality of the stone taps in Kathmandu city and reported that bacteriologically all the samples had faecal contamination in both seasons, hence water from all the stone taps were unfit for drinking and Prasai (2002) assessed 132 water samples from Kathmandu City and reported that 82.6% and 92.4% of samples crossed WHO guideline value for total bacterial count and coliform count. All the above mentioned studies suggest drinking water of Kathmandu city is a serious public health issue for Kathmanduties.

6.1.3 Enteric bacteria isolated from water samples

Ten different kinds of enteric bacteria were isolated from the total contaminated samples. Among the isolates, *Citrobacter* spp. (26.22%) was found to be maximum followed by *E. coli* (25%), *Enterobacter* spp. (20.73%), *Shigella* spp. (8.54%), *Proteus vulgaris* (7.93%) *Pseudomonas aeruginosa* (3.66%), *Salmonella paratyphi* (3.05%), *Klebsiella* spp. (2.44%), *Proteus mirabilis* (1.83%) and *Salmonella typhi* (0.61%).

Sharma (1993) also isolated enteric pathogens viz. *E. coli, Proteus* spp., *Salmonella* spp. and *Klebsiella* spp. from water samples. Thapa (1997) also isolated *E. coli, Salmonella* spp. and *Klebsiella* spp.. Maharjan (1998) also isolated *Enterobacter* spp. as the predominant bacteria followed by *E. coli* > *Citrobacter* spp. > *Salmonella* spp. and others. Similarly Prasai (2002) isolated 238 enteric bacteria from different sources. Percentage recovery of *E. coli* (26.4%) was found to be maximum followed by *Enterobacter* spp. (25.6%), *Citrobacter* spp. (22.6%), *Pseudomonas aeruginosa (*6.3%), *Klebsiella* spp. (5.4%), *Shigella* spp. (3.78%), *Salmonella typhi* (3.3%), *Proteus vulgaris* (2.9%), *Serratia* spp. (2.52%) and *Vibrio cholerae* (0.84%).

Thus, deteriorating water quality is the major problem and it has created serious threat to human health and environment. The quality of water has deteriorated due to the poor management and no monitoring of water quality. Bacteriological pollution of drinking water supplies may be either due to the failure of the disinfections of the raw water at the treatment plant or because of the infiltration of contaminated water (sewage) through cross- connection and leakage points. The result clearly showed that the quality of the water consumed is critical in controlling infectious diseases and other health problems. As water may contain disease-causing microorganisms (pathogens) or chemicals that are toxic, water quality is a key factor in promoting and maintaining good health. Provision of clean and safe drinking water is a prerequisite for public health and well being.

The greatest problem continues to be the microbial contamination of drinking water supplies (especially faecal contamination) plus chemical contaminants notably arsenic is of increasing concern. It is a tragedy that infants and young children are the innocent victims of the failure to make safe drinking water and basic sanitation services. Lack of drinking water and sanitation kills about 4500 children a day and sentences their siblings, parents and neighbours to sickness. In Nepal, morbidity and mortality rates from water borne disease are considered high particularly among children below the age of five. Improvements (safe water supply and of adequate means of sanitation) bring immediate and lasting benefits in health, dignity, education, productivity and income generation. The conservation of water sources is very important to provide safe water. As far as possible, water sources must be protected from contamination by human and animal waste, which can contain a variety of bacterial, viral, protozoan and helminthes parasites. The control of drinking water quality in distribution networks remains a major challenge in urban areas. The protection of sources, treatment and distribution management are all-critical strategies in maintaining and improving piped water supplies. Prevention of the disease is therefore a matter of providing safe drinking water.

Correlation among different parameters for water samples:

The conductivity is a measurement of the ions in the water. The ions include the nitrates, calcium, magnesium, chloride, iron, arsenic and other soluble ions. Water samples that had higher chloride content, iron values, hardness, nitrate and ammonia values had obviously

higher readings of conductivity.The positive correlation between turbidity and iron content of water may be attributed to precipitation of iron salts causing turbidity.

The suggested relationships and correlations between temperature, pH, turbidity, and total coliforms are not conclusive. The number of samples taken is too small to perform a comprehensive statistical analysis of the data. The trends are general indications of possible relationships that would require additional sample data to be tested. In addition, there may be other factors that affect the results including temperature, and other substances in the water.

6.1.4 Medicinal plants

The wealth of medicinal plants in Nepal may be considered as one of the important natural resources for the economy of this Himalayan Kingdom. Many kinds of plant grow here and a large number of them have been used for medicinal purposes. In the present study, an attempt has been made to evaluate the antimicrobial properties of eight important medicinal plants traditionally used in various therapies in the Himalaya.

6.1.4.1 Selection, identification and processing of the plants

The selection of plant was done on the basis of its use for diseases like cholera, diarrhoea, dysentery and enteric fever as cited in different literature.

Samples were properly labeled and left for shade drying as drying in sunlight may cause loss of some active compounds. Large plant part if extracted without grinding results in poor percentage yield on extraction; so the dried plant samples were ground to fine powder.

6.1.4.2 Extraction of medicinal plants

Commonly, methanol, ethanol and other alcohols and occasionally acetone, chloroform and petroleum ether were found to be used for extraction purposes. However, in most of the cases methanolic, ethanolic and other alcohol extracts were found to be most effective. (Timsina, 2003). Water, the universal solvent, was also found to be used from time to time. But aqueous extracts was found less effective than the alcoholic extracts as it is less effective for non polar organic compounds.

6.1.4.3 Screening and evaluation of antimicrobial activity

Out of eight medicinal plants tested against 10 different bacterial species in the present study, 4 plants namely *Punica granatum, Woodfordia fruticosa, Psidium guajava and Syzygium cumini* showed antimicrobial activity against all the tested organisms. The screening process just indicates whether or not a compound has antimicrobial activity against the test bacteria, i.e. the result is either (+) ve or (-) ve. It doesn't indicate the extent of the effect i.e. potency of sample cannot be determined by screening alone. A substance may demonstrate a wide spectrum of activity but have low potency. Such a substance has no utility practically and development of such product is not economically feasible. Therefore the antimicrobial activity must also be quantitatively assayed. This has been done here by two different methods. Agar well diffusion and two fold serial dilution. The parameters were measured accordingly different zone of inhibition and minimum bactericidal concentration (MBC).

Antimicrobial substance applied in the agar well diffuses readily in concentric circles and inhibits or kills the microorganisms that are susceptible. This effect, manifested by a zone of clearing, is observed upto a point where the decreasing concentration of the diffusing antimicrobial substance is still sufficient to inhibit or kill the organism. Beyond the point, the concentration is insufficient and hence, growth starts. Thus by measuring the zone of inhibition of the antimicrobial for various organisms, we can estimate the degree of susceptibility or sensitivity.

The cidal activities of medicinal plants like *Punica granatum, Woodfordia fruticosa, Psidium guajava* and *Syzygium cumini* are due to the active constituents present in them. The compounds like ellagitannins and alkaloids in the rind of fruit *Punica granatum*, flavonoids and tannins in leaf of *Syzygium cumini*, comarins, essential oils, flavonoids, triterpenes and ellagitannins in leaf of *Psidium guajava*, tannins, flavonoids, anthraquinone glycosides and polyphenols in *Woodfordia fruticosa* have been reported (Nascimento et al 2000). Other four plants namely *Mimosa pudica, Acorus calamus, Aegle marmelos* and *Anethum sowa*, though were selected on the basis of their use in common diseases like diarrhoea, dysentery, fever etc, they didn't show antimicrobial activity against enteric bacteria isolates. This can be due to the inability of water to dissolve the active components of alcoholic extracts of the plants during preparation of working solution

It was revealed from the result that each medicinal plant shows different degree of inhibiton against different microorganisms. The maximum zone of inhibition was observed in case of *Proteus mirabilis* (31mm) due to the action of *Syzygium cumini* and minimum was against *Pseudomonas* spp. (10mm) shown by *Psidium guajava*.

The diameter of zone of inhibition produced depends on several factors broadly classified as extrinsic and intrinsic parameters. The extrinsic parameters like pH of the medium, period and temperature of incubation, volume of the well, concentration of plant extracts and size of inoculums can be fixed and standardized during experiment, hence no error results due to extrinsic factors. However, intrinsic factors such as nature of medicinal plants including its components, solubility and diffusing property are predetermined. Due to variable diffusibility, the antibacterial with very high potency may not demonstrate a ZOI commensurate to its efficacy.

Actually the concentration of antimicrobial substance at the periphery of ZOI is the minimum concentration required to inhibit or kill the bacteria. But it cannot be said whether the growth has been just inhibited or the bacteria has actually been killed as well. This further necessitates MBC (minimum bactericidal concentration) determination. Therefore, MBC values have also been computed here with two fold serial dilution method. MBC is the lowest concentration of antimicrobial substance required to produce a sterile culture (Cheesbrough, 1993).

The bacteria were inoculated in a series of tubes with decreasing concentration of the antimicrobial substance. After proper incubation, the results were compared with +ve and –ve growth control tubes. The tubes that showed no growth were sub-cultured onto nutrient agar devoid of the antibacterial substance. The minimum concentration that failed to show growth in nutrient agar plate was taken as MBC value.

For *E .coli*, *Enterobacter* spp. and *Shigella* spp, MBC value of 50 mg/ml was obtained with *Punica granatum, Woodfordia fruticosa, Psidium guajava* and *Syzygium cumini.* For *citrobacter* spp, MBC value of 50 mg/ml was obtained with *Woodfordia fruticosa, Psidium*

guajava whereas *Punica granatum and Syzygium cumini* had cidal activity at a concentration of 25 mg/ml. *Woodfordia fruticosa, Psidium guajava and Syzygium cumini* had cidal activity at a concentration of 50 mg/ml *for Klebsiella* spp and *Punica granatum* showed lethal effect at 6.25mg/l

For *Salmonella typhi, Woodfordia fruticosa* was the most lethal requiring as low as concentration as 1.56 mg/ml. *Punica granatum* and *Syzygium cumini* had cidal activity at a concentration of 3.12 mg/ml where as *Psidium guajava* could showed the activity only at 6.25 mg/ml. *Punica granatum, Woodfordia fruticosa, Psidium guajava and Syzygium cumini* showed lethal effect against *Salmonella paratyphi* with MBC value of 1.56 mg/ml, 25 mg/ml, 3.12 mg/ml, and 0.39 mg/ml respectively.

For *Proteus mirabilis* 12.5mg/l was MBC value shown by *Punica granatum*, *Psidium guajava* and *Syzygium cumin*i. For *Proteus vulgaris* MBC value of 6.25 mg/ml, 1.56 mg/ml, 1.56 mg/ml, and 25 mg/ml were shown by *Punica granatum, Woodfordia fruticosa, Syzygium cumini, and Psidium guajava* respectively. For *Pseudomonas* spp. MBC value of 50 mg/ml was obtained with *Punica granatum, Woodfordia fruticosa, Psidium guajava* and 25 mg/l with *Syzygium cumini*.

The above data showed that *Salmonella typhi, Salmonella paratyphi*, *Proteus vulgaris* and *Proteus mirabilis* were more susceptible than remaining tested organisms. The zone of inhibition (ZOI) and minimum bactericidal concentration (MBC) are two different attributes and there is absence of linear relationship between ZOI and MBC values.

6.2 Conclusion

The present study disclosed the physicochemical and bacteriological contamination of different water sources in Kathmandu metropolitan areas. Besides coliform contamination, the concentration of iron and ammonia was high in some of the water samples. Some water samples were also found to have arsenic contamination indicating serious health threat to the people of Kathmandu. So, the study has pointed out that the drinking water quality of city water supply has not been improved and traditional sources like stone spouts and tube well

water are also not free from contamination. Such circumstances are responsible for spreading water borne outbreaks. The waterborne diseases are closely related with the conditions of living and environmental sanitation in the community. So, it can be effectively controlled by appropriate water management and safe disposal of excreta. The use of the plants to heal diseases, including infectious one, has been extensively applied by many people. Data from the literature as well as the result reveal the great potential of plants for therapeutic treatment.

CHAPTER-VII

7 SUMMARY AND RECOMMENDATIONS

7.1 Summary

1. A total of one hundred and fourteen samples of drinking water were randomly collected from different areas of Kathmandu metropolitan city. All water samples were analysed for physicochemical and microbiological parameters to assess the drinking water quality. Source wise distribution of samples showed the equal number of samples from each sources namely stone spouts, taps and tube wells.

2. The physicochemical analysis of water reflects the following results. The pH of 14.91 % of water samples were found to lie below WHO guideline value. Similarly 24.56%, 26.32% and 31.58% of water samples were found to exceed WHO guideline value for conductivity, turbidity and iron content respectively. Hardness and the chloride content of all water samples were within the guideline value whereas 22.81% of samples crossed ammonia permissible level. The nitrate content of all samples except one tube well sample was found within permissible level. Similarly, seven water samples (6.14%) exceeded the WHO guideline for arsenic level in the study.

3. The bacteriological analysis of water samples revealed the presence of total coliform in 90.35% of total samples (tube well-97.37 %, tap-73.68% and stone spout-100 %). Ten different kinds of enteric bacteria were isolated from the total contaminated samples. Citrobacter spp. (26.22%) being the predominant.

4. Eight different medicinal plants were screened and evaluated for their antimicrobial activity against the enteric bacteria isolated from water on the basis of their common use among the different ethnic groups for common disorder. Among the selected medicinal plants, *Punica granatum* gave the highest yield (25.30%) followed by *Woodfordia fruticosa (24.8%), Mimosa pudica (*24.2%), *Syzygium cumini (*21.40%), *Psidium guajava*

(16.87%), *Acorus calamus* (15.50%), *Aegle marmelos* (14.14%) and *Anethum sowa* (7.59%).

5. All enteric bacteria isolated from drinking water were tested for the antimicrobial assay of some medicinal plants. Out of eight, four medicinal plants (*Punica granatum, Woodfordia fruticosa, Psidium guajava* and *Syzygium cumini)* were found to be effective against all enteric bacteria.

6. The medicinal plants showed different degree of inhibiton against different microorganisms. The maximum zone of inhibition was observed in case of *Proteus mirabilis* (31mm) due to the action of *Syzygium cumini* and minimum was against *Pseudomonas* spp. (10mm) shown by *Psidium guajava*. The minimum bactericidal concentration (MBC) of these plant extracts found against *Salmonella typhi, Salmonella paratyphi, Proteus mirabilis* and *Proteus vulgaris* were lower (0.39-25mg/ml) so are more susceptible whereas the plants showed lethal effect against *Pseudomonas* spp, *Citrobacter* spp, *Enterobacter* spp, *E. coli, Shigella* spp. and *Klebsiella* spp. at MBC value of around 25-50mg/ml.

7.2 Recommendations

- This study is limited to Kathmandu Metropolitan City (KMC) areas. Thus the findings may not generalize the whole Kathmandu district. Water quality testing should also be done in other parts of Kathmandu Valley, which are not covered by the present study.

- Awareness building and motivational programs regarding water pollution and its possible health impacts should be organized by NWSC and other concerned agencies to make people aware and conscious about the quality of drinking water.

- People should be trained regarding the simple and cheap techniques of water treatments such as boiling, filtering and chlorinating.

- In this study, qualitative or semi-qualitative method for detection of arsenic was used. Therefore, sophisticated instrument like atomic absorption spectrophotometer should be used for quantitative analysis.

- In this study, only 8 medicinal plants have been assayed due to constraint of time. Antimicrobial activities of many other traditionally used plants should also be evaluated.

- Phytochemical screening of medicinal plants should be performed so that the major groups of compounds of biological interest present in the particular plant will be known.

- Only *in-vitro* tests have been performed in this study. Further researches should be directed towards *in-vivo* tests to evaluate the antimicrobial activity of medicinal plants of Nepal.

CHAPTER VII

8. REFERENCES

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APPENDIX – 1

1.0 List of materials

1.1) Equipment

1. Autoclave
2. Conductivity meter
3. Electric balance
4. Grinder
5. Incubators
6. Laminar airflow Cabinet
7. Membrane filter apparatus
8. Microscope
9. Nephelometer
10. pH meter
11. Refrigerator
12. Rotary vacuum evaporator
13. Soxhlet extractor
14. Spectrophotometer
15. Vortex shaker
16. Water bath shaker
17. Water distillation plant

1.2) Microbiological media

MacConkey Agar (Hi-media)
M-endo Agar (Hi-media)
Muller Hinton Agar (Hi-media)
Nutrient Agar (Hi-media)
Nutrient Broth (Hi-media)
Peptone (Hi-media)
Salmonella-Shigella Agar (Hi-media)
Selenite F Broth (Hi-media)
Thiosulphate Citrate Bile Salt Sucrose Agar (Hi-media)
Xylose Lysine Deoxycholate Agar (Hi-media)

1.3) Biochemical media

MR-VP Broth (Hi-media)
Simmon'citrate Agar (Hi-media)
Triple sugar iron agar (Hi-media)
Urea Agar base (Hi-media)
Hugh-Leifson's Agar (Hi-media)
Sulphide Indole Motility medium (Hi-media)

1.4) Glassware

Beakers	Glass rods	Pipette
Burette	Graduated cylinders	Round Bottom Flasks
Condensers	Micropipettes	Reagent bottles
Conical flasks	Slides	Screw capped test tubes
Funnels	Petridishes	Test tubes etc

1.5) Miscellaneous

Aluminum foil	Immersion oil	Sampler (sample collecting bottles)
Blotting paper	Cotton-swab	Lens/Tissue paper for microscopy
Cotton role	Inoculating loop	Soap (Dettol) / Detergent
Cork borer	Forceps	Transport tray etc
Dropper	Sticker	

1.6) Chemicals/Reagents

Ammonium-chloride	Potassium permanganate
Ammonium acetate	Potassium chromate
Acetic acid	1, 10-phenanthroline
α-Naphthol	Saffranin
Ammonia-buffer	Sodium hydroxide
Ammonium hydroxide	Silver nitrate
Barium Chloride	Sulfuric acid
Conc. Hydrochloric acid	Tetramethyl-p-phenylenediamine dihydrochloride
Crystal violet	
Disodium salt of EDTA	
Erichrome Black T	
Ethanol	
Ferrous-ammonium sulfate	
Gram's iodine	
Hydrogen peroxide	
Hydroxylamine hydrochloride	
Iodine	
Iso-amyl alcohol	
Kovac's reagent	
Lead-acetate trihydrate	
Lysol, Mercuric iodide	
Methyl red	
Paraffin	
Potassium hydroxide	
Potassium iodide	

APPENDIX -2

Morphological and Biochemical characteristics of enteric bacteria

Gram reaction	-	-	-	-	-	-	-	-	-	-	-
Shape	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	Curved rod
Catalase reaction	+	+	+	+	+	+	+	+	+	+	+
Oxidase reaction	-	-	-	-	-	-	-	-	-	-	+
Glucose OF medium	F	F	F	F	F	F	F	F	F	O	O
MR Test	+	-	+	-	+	+	+	+	+	-	+
VP Test	-	+	-	+	-	-	-	-	-	-	-
Citrate Test	-	+	+	+	-	-	-	+/-	+/-	+	-
Indole Test	+	-	-	-	-	-	D	-	+	-	+/-
Urease Test	-	+	-	-	-	-	-	+	+	-	-
Glucose fermentation to acid or to acid and gas	+	+	+	+	+	+	+	+	+	+	+
Lactose (acid)	+	+	+	+	-	-	-	-	-	-	-
H_2S Production	-	-	+	-	+	-	-	+/-	+/-	-	-
Motility	+	-	+	+	+	+	-	+	+	+	+
Escherichia coli	*										
Klebsiella sp.		*									
Citrobacter sp.			*								
Enterobacter sp.				*							
Salmonella typhi					*						
Salmonella paratyphi						*					
Shigella sp.							*				
Proteus mirabilis								*			
Proteus vulgaris									*		
Pseudomonas sp.										*	
Vibrio cholerae											*

APPENDIX-3

List Of Medicinal Plants

S.N	Local Name	Botanical Name	Family	Part used	Month of collection	Location /District
1	Saunp	*Anethum sowa*	Umbelliferae	seeds	April	Market /Kathmandu
2	Bel	*Aegle marmelos*	Rutaceae	Fruit	May	Rudrapipal/Baglung
3	Anar	*Punica granatum*	Punicaceae	rind of fruit	June	Market /Kathmandu
4	Dhanyero	*Woodfordia fruticosa*	Lythraceae	leaves	May	Godawari/Lalitpur
5	Lajjawati	*Mimosa pudica*	Febaceae	leaves	June	Khumaltar/Lalitpur
6	Guava	*Psidium guajava*	Myrtaceae	leaves	June	Thimi/Bhaktapur
7	Jamun	*Syzygium cumini*	Myrtaceae	leaves	June	Chyashal/Lalitpur
8	Bojho	*Acorus calamus*	Araceae	rhizome	May	Godawari/Lalitpur

APPENDIX-4

Turbidity standard

Turbidity standard is prepared by pouring 0.6 ml of 1 % (10 g/litre) solution of barium chloride dihydrate into a 100 ml graduated cylinder, and filling to 100 ml with 1 %(10 ml/litre) sulphuric acid. The turbidity standard solution should be placed in a tube identical to the one used for the broth sample. It can be stored in the dark at room temperature for 6 months, provided it is sealed to prevent evaporation.

Source: Basic laboratory Procedure in Clinical Bacteriology. World Health Organization. Geneva (1991)

Actual Concentration Extracts During Two Fold Serial Dilution:

Code name of test tube	Concentration	
	mg/ml	µg/ml
-ve control	100.00	100,000.0000
1st	50.00	50,000.0000
2nd	25.00	25,000,0000
3rd	12.50	12,500.0000
4th	6.25	6250.0000
5th	3.12	3125.0000
6th	1.56	1562.0000
7th	0.78	781.0000
8th	0.39	390.0000
9th	0.19	195.3125
10th	0.09	97.6565
+ve control	0.00	0.0000

APPENDIX-5

WHO standards for Drinking Water

S.N	Parameters	Unit	WHO Guideline Value
1	***Physical Parameters***		
i.	Temperature	°C	
ii.	pH		6.5-8.5
iii.	Conductivity	µs/cm	500
iv.	Turbidity	NTU	5
2	***Chemical Parameters***		
i.	Hardness	mg/l	500
ii.	Chloride	mg/l	250
iii.	Iron	mg/l	0.3
iv.	Ammonia	mg/l	1.5
v.	Nitrate	mg/l	50
vi.	Arsenic	mg/l	0.01
3	***Bacteriological Parameter***		
i.	Total Coliform Count	cfu/100ml	0