

STUDY ON ANTIBACTERIAL ACTIVITY OF COMMON MEDICINAL PLANTS USED TO TREAT DIABETES IN BANGLADESH

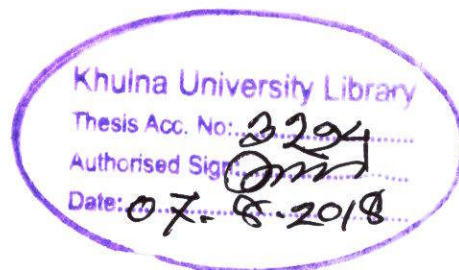
A Thesis

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*DEDICATED TO MY
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TITLE

STUDY ON ANTIBACTERIAL ACTIVITY OF COMMON MEDICINAL PLANTS USED TO TREAT DIABETES IN BANGLADESH

DECLARATION

This thesis report is the original research work of the authors, except as otherwise stated. The material presented has not submitted either in whole or in part for a degree for this or any other university. The findings of the research has not been/will not be published anywhere without the written concurrence of the concerned supervisor. Legal action would be taken in any case of infringement regarding the declaration.


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The Author

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ABSTRACT

Medicinal plants, besides having therapeutic values against diabetes mellitus may have other pharmacological actions. To know whether common antidiabetic plants in Bangladesh possess antibacterial activity, methanol extracts of them were tested against six pathogenic bacteria. Among the extracts, *Coccinia indica* (leaves), *Gymnema sylvestre* (Bark), *Swertia chirata* (whole part) and *Syzygium cumini* (seed) showed strong inhibition against *Salmonella typhi*, *S. paratyphi* A, *Staphylococcus aureus* and *Shigella dysenteriae* in disc diffusion test at the concentration of 400 µg/disc. No extract inhibited the growth of *Vibrio cholerae*. After that, extracts of *C. indica*, *G. sylvestre* and *S. cumini* were subjected to evaluate their IC₅₀ values. The lowest IC₅₀ value was found in *C. indica* against *S. paratyphi* A. Since inhibitory actions of *C. indica*, *G. sylvestre*, *S. chirata* and *S. cumini* were found efficient, they had been fractionated into five fractions namely hexen, diethylether, chloroform, ethylacetate and methanol. Among the fractions, methanol fraction of *S. cumini* effectively inhibited the growth of all four bacteria at the concentration of 400 µg/disc, whereas, *S. paratyphi* A was inhibited by *C. indica* and *S. typhi* by both *G. sylvestre* and *S. chirata*. However, the methanol fractions showed lesser inhibition than that of methanol extracts against the bacteria which entails the idea of synergistic action. Since methanol fractions of the four plants were the most potent their IC₅₀, MIC and MBC values were determined. Altogether, the results showed that among the common antidiabetic plants, *S. cumini* had potent antibacterial activity that might be used in treating both diabetes and bacterial infections.



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ABBREVIATION

CFU	Colony Forming Unit
DM	Diabetes mellitus
DMSO	Dimethyl sulphoxide
GI	Gastro-intestinal
IC ₅₀	Inhibitory Concentration 50
ICC	Intestinal Cells of Cajal
LPS	Lipopolysaccharide
MBC	Minimum Bactericidal Concentration
MeOH	Methanol
mg	Milligram
MIC	Minimum Inhibitory Concentration
mL	Milliliter
mm	Millimeter
NA	Nutrient agar
NB	Nutrient broth
NIDDM	Non-insulin dependent diabetes mellitus
OD	Optical Density
SD	Standard Deviation
Tet.	Tetracyclin
UTI	Urinary Tract Infection
µg	Microgram
µL	Microliter
µm	Micrometer

Chapter One

INTRODUCTION

INTRODUCTION

Medicinal plants have been used by mankind since the beginning of human civilization for their enormous therapeutic value. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. The plant-based, traditional medicine systems continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi et al., 2007).

The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of health has been widely observed (UNESCO, 1996). Modern pharmacopoeia still contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants. Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well being and the bioprospecting of new plant-derived drugs (Lucy and Edgar, 1999). The ongoing growing recognition of medicinal plants is due to several reasons, including escalating faith in herbal medicine (Kala, 2004). The medicinal properties of plants could be based on the antioxidant, antimicrobial, antipyretic effects of the phytochemicals in them (Cowman, 1999; Adesokan et al., 2008). According to world ethnobotanical information reports, almost 800 plants may possess antidiabetic potential (Alarcon-Aguilara et al., 1998) among them lots of herbs are now being used in the management of diabetes mellitus (Grover and Yadav, 2004). Diabetes is a chronic disorder in metabolism of carbohydrates, proteins, and fat due to absolute or relative deficiency of insulin secretion with/without varying degree of insulin resistance (Devlin 1997; Barar 2000). Also, it may be defined as a disease where the body either produces little insulin/ceases to produce insulin, or becomes progressively resistant to its action (Ranjan and Ramanujan, 2002). It has now become an epidemic with a worldwide incidence of 5% in the general population. The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in the year 2025 (Torben, 2002). Diabetes results in dysfunction of the entire gastro-intestinal (GI) tract, where 100 trillion microorganisms, encompassing up to thousand of species at an average concentration of 10^{14} per ml and weighing in average 1.5 kg can be found in the human (Sellin and Chang, 2008). GI symptoms are

frequently elicited in patients attending diabetic clinics. As many as 75% of patients visiting diabetes clinics are reported to have significant GI symptoms. The entire GI tract can be affected by diabetes from the oral cavity and esophagus to the large bowel and anorectal region. Thus, the symptom complex that may be experienced can vary widely. Common complaints may include dysphagia, early satiety, reflux, constipation, abdominal pain, nausea, vomiting, and diarrhea. Many patients go undiagnosed and under-treated because the GI tract has not been traditionally associated with diabetes and its complications. Both acute and chronic hyperglycemia can lead to specific GI complications. Diabetes is a systemic disease that may affect many organ systems, and the GI tract is no exception. As with other complications of diabetes, the duration of the disorder and poor glycemic control seem to be associated with more severe GI problems. Patients with a history of retinopathy, nephropathy, or neuropathy should be presumed to have GI abnormalities until proven otherwise (Bytzer et al., 2001; Vinik et al., 2003). Studies also indicate that there are likely to be multiple etiologic factors behind GI symptoms. For example, autonomic neuropathy was identified as being the cause of many GI symptoms (Mjornheim et al., 2003). Causes of gastrointestinal problems in patients with diabetes can include certain metabolic insults that result in smooth muscle atrophy or in the transformation of one cell type to another (He et al., 2001). The pacemaker cells of the intestinal tract—the interstitial cells of Cajal (ICCs)—seem to be disrupted or altered in some way in diabetes. Other potential causes of intestinal dysfunction include ischemia, hypoxia, and mitochondrial dysfunction (Leininger et al., 2008). Cumulatively, these metabolic and anatomic changes cause abnormalities in peristalsis, reflexive relaxation, sphincter tone, vascular flow and intestinal segmentation. These abnormalities manifest themselves clinically as dysphagia, gastroparesis, constipation, diarrhea, intestinal pseudo-obstruction and anal incontinence. Decreased physical activity, increasing obesity, stress and changes in food consumption have been implicated in this increasing prevalence in the past two decades (Atkinson et al, 1983; Feldmen and Schiller 1983; Turnbaugh et al., 2007).

Despite advances in the knowledge of the etiology, pathophysiology, and management of diabetes mellitus, infections rank as the most important complication of the disease (Louie et al., 1993). Impaired leukocyte function, associated vascular disease, poor control of diabetes, and various host factors all contribute to the pathogenesis of infection

in the diabetic patient (Rayfield et al., 1982; Sentochnik and Eliopolous, 1995). Patients with diabetes seem to be at particularly high risk for infection with certain microorganisms. For example, in a group of nonpregnant adults with group B streptococcal bacteremia, the prevalence of diabetes was found to be 27.5 percent (Farley et al., 1993). A disproportionately high incidence (30 to 60 percent) of diabetes has been reported in several series of patients with *Klebsiella* infections, including bacteremia (Leibovici et al., 1991), liver abscess (Wang et al., 1998), endophthalmitis (Chee and Ang, 1995) and thyroid abscess (Li et al., 1997). Diabetes has been identified as a risk factor for infection with *Salmonella enteritidis* (Telzak et al., 1991). In several studies in the first half of the 20th century, the incidence of tuberculosis among persons with diabetes was found to be three or four times as high as in the general population (Joshi et al., 1999). Lower extremity infections are a serious cause of morbidity in patients with diabetes mellitus. These infections are responsible for 20% of all hospital admissions in diabetic patients (Sapico, 1989). Diabetes is the major cause of non-traumatic limb amputation (Gibbons and Eliopoulos, 1984). Several immune defense mechanisms are defective in diabetic patients. Among these are a decrease in leukocyte chemotaxis (Mowat and Baum, 1971), phagocytosis (Bybee and Rogers, 1964) and intracellular killing (Tan et al., 1975). Microbiologically, diabetic foot infections are generally polymicrobial. Among the most frequently isolated microorganisms from the lesions are *Staphylococcus aureus*, group B streptococci, *Enterococci*, anaerobic bacteria including *Bacteroides fragilis* and some enteric gram-negative organisms (Kajetan et al., 1995; Goldstein et al., 1996). The majority of infections in diabetic patients are localised in the urinary tract (Carton et al., 1992). The bacteria isolated from diabetic patients with a Urinary Tract Infection (UTI) are similar to those found in non-diabetic patients with a complicated UTI (Nicolle, 2001). As in uncomplicated UTIs, *E. coli* causes the majority of infections. However, other strains are relatively more frequently cultured in these patients. For example, one study reported *E. coli* to be the causative uropathogen in 47% of the UTIs in diabetic patients and in 68% of the UTIs in non-diabetic patients (Lye et al., 1992). Non-*E. coli* uropathogens found in patients with diabetes mellitus, include *Klebsiella spp*, *Enterobacter spp*, *Proteus spp*, Group B *Streptococci* and *Enterococcus faecalis* (Wheat, 1980; Zhanel et al., 1991; Zhanel et al., 1995).

Diabetes is being projected as the World's main disabler and killer in the next 25 years (Edwin et al., 2006). Many plants have been used for the treatment of diabetes mellitus in traditional systems of medicine throughout the world. Indeed, along with dietary measures, plant preparation formed the basis of the treatment of the disease until the introduction of insulin in 1922 (Bailey and Day, 1989; Swanston-Flatt et al., 1991) and a range of plants and phytochemicals with reputed hypoglycaemic effects have been scientifically investigated (Perl, 1988; Handa et al., 1989; Day, 1990; Marles and Farnsworth, 1995). There is increasing demand by patients to use the natural products with antidiabetic activity. In recent times there has been renewed interest in the plant remedies. Plants hold definite promises in the management of diabetes mellitus. In spite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease. In general, there is very little biological knowledge on the specific modes of action in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc. that are frequently implicated as having antidiabetic effects and might have other effect like antimicrobial activity. The research for alternate remedies (from the plant kingdom) for diabetes mellitus will continue all over the world as the disease poses many challenges not only to the physician but also to the researcher (Resmi 2001; Grvoer et al., 2002; Malviya et al., 2010).

Bangladesh is full of medicinal plants, which are used by the people for the treatment of various diseases even at this modern era. Bangladesh, together with India, constitutes the largest part of South Asian plant genetic diversity. However, Bangladesh have about 5,000 species of higher plants of which over 500 have ethnomedical value. Recently, 546 species have been identified as having medicinal and therapeutic use, of which 257 in effective remedies for diarrhoea and 47 for diabetes (Haque, 2004). In Bangladesh about 5 million people are affected with diabetes and in recent years the popularity of complementary medicines has increased. Dietary measures and traditional plant therapies as prescribed by Ayurvedic and other indigenous systems of medicine have been well adapted in Bangladesh. Some important plants used to treat diabetes are *Allium sativum*, *Alstonia scholaris*, *Catharanthus roseus*, *Cephalandra indica*, *Clerodendrum viscosum*, *Coccinia grandis*, *Cuscuta reflexa*, *Drynaria quercifolia*, *Enhydra fluctuans*, *Terminalia chebula*, *Lagerstroemia speciosa*, *Lannea coromandelica*, *Momordica charantia*,

Phyllanthus emblica, *Scoparia dulcis*, *Syzygium aqueum*, (Rahman et al., 2002; Rahmatullah et al., 2012).

Medicinal plants produce bioactive compounds used mainly for medicinal purposes. These compounds either act on different systems in man, and/or act through interfering in the metabolism of microbes infecting them. The microbes may be pathogenic or symbiotic. In either way the bioactive compounds from medicinal plants play a determining role in regulating host-microbe interaction in favour of the host (Malviya et al., 2010). So the identification of bioactive compound in plants, their isolation, purification and characterization of active ingredients in crude extracts by various analytical methods is significant.

Therefore, in the present study, we have investigated the antibacterial activity of the crude extracts of antidiabetic plants commonly used in Bangladesh to treat diabetes.

Objectives:

The objectives of the research work are mentioned below-

- to know whether the common antidiabetic medicinal plants in Bangladesh possess antibacterial activity
- to know whether they inhibit the growth of common pathogenic bacteria which cause diseases in human
- to search out medicinal plant(s) which is potent against bacteria as well as diabetes
- to know the antibacterial active fraction(s) in the antidiabetic plants
- to clarify the antibacterial component(s) in antidiabetic medicinal plants

Chapter Two

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Diabetes mellitus (DM) is the commonest endocrine disorder that affects more than 100 million people worldwide (6% of the population) (WHO/Acadia, 1992). It is caused by deficiency or ineffective production of insulin by pancreas which results in increase in concentrations of glucose in the blood. It is found to damage many of the body systems, particularly the blood vessels and nerves (Nagappa et al., 2003). Both type 1 and type 2 diabetes mellitus can affect a patient's entire gastrointestinal (GI) tract, from the esophagus to the anus. The symptoms that often accompany the GI effects of diabetes mellitus cause a great deal of misery for patients, as well as frustration for the physicians taking care of them. Because GI symptoms are common in the general population, physicians may have difficulty determining whether diabetes is the root problem (Sellin and Chang, 2008).

In the past, physicians assumed that hyperglycemia leads to autonomic neuropathy, which leads to the GI abnormalities. In addition, physicians thought that patients must have diabetes for 10 years or more before GI symptoms occur. Recent findings, however, call both of these premises into question. The most common GI problems encountered in clinical practice among patients with diabetes are constipation, diarrhea, abdominal pain, nausea and vomiting. Several other problems may be seen from time to time (Vinik et al., 1989). Growing evidence suggests factors other than dietary habits can modulate gut microflora and that the 1st years of life have a crucial impact on the individual's gut microbiota composition (Kalliomäki et al., 2008). Microorganisms occur along the whole length of the human alimentary tract with population numbers and species distribution characteristic of particular regions of the gut (Macfarlane et al., 1997). Through the microflora, the colon is capable of complex hydrolytic digestive functions (Cummings and Macfarlane, 1991). This involves the breakdown of dietary components, principally complex carbohydrates, but also some proteins, that are not hydrolysed nor absorbed in the upper digestive tract (Macfarlane et al., 1992). Carbohydrate availability subsequently diminishes as dietary residues pass from the proximal colon to the transverse and distal bowel. For persons living on Western-style diets, the microbial biomass makes up over 50% of colonic contents. There are more than 500 different culturable species of indigenous bacteria present in the adult large intestine comprising around 10^{12} bacteria per gram dry weight (Moore et al., 1978; Simon and Gorbach, 1984). In very general terms, intestinal bacteria can be divided on the basis of

whether they can exert health promoting, benign or potentially harmful activities in their host (Gibson and Roberfroid, 1995). The most obvious pathogens are strains of *Escherichia coli* and *Clostridia*. Pathogenic effects include diarrhoeal infections and putrefaction whereas beneficial aspects may be derived simply by improved the digestion/absorption of essential nutrients. This leads towards a consideration of factors that may influence the flora composition in a manner than can impact upon health (Gibson et al., 1997). It is now known that bacterial activity in the human colon is involved in a number of disease states. The large intestine can harbor pathogens that are either part of the resident flora or exist as transient members. Attachment and overgrowth of the pathogens generally results in acute diarrhoeal infections, however more chronic forms of intestinal disease also occur (Gibson et al., 1997; Gionchetti et al., 2000). These include inflammatory bowel diseases (ulcerative colitis and Crohn's disease) (Chadwick and Anderson, 1995), colon cancer (Rowland, 1988; Rumney et al., 1993) and pseudomembranous colitis (Duerden et al., 1995). To varying extents, each has been linked into microflora composition and activities, and thereby diet as this provides the major source for their growth. Diet and nutritional status are amongst the most important modifiable determinants of human health. Furthermore, a change in feeding habits is most likely the most prevalent factor susceptible to induce metabolic diseases. Many studies have reported direct links between diet and the structure of the gut microbiome in mouse models. One recent example observed that microbiome structure rapidly shifts in response to a change from a low-fat, plant-based diet to a high-sugar, high-fat diet, modifying both the available metabolic pathways and actual gene expression (Cani et al., 2008, Turnbaugh et al., 2009). Furthermore, the change in feeding habits was associated with an increased intestinal permeability to lipopolysaccharides (LPS) leading to a state of insulin resistance (Cani et al., 2007, Turnbaugh et al., 2009). This latter study further demonstrated that the change in intestinal microbiota was also associated with a different susceptibility to antibiotic treatment (Turnbaugh et al., 2009).

The management of diabetes is a global problem until now and successful treatment is not yet discovered. There are many synthetic medicines developed for patients, but it is the fact that it has never been reported that someone had recovered totally from diabetes (Li et al., 2004). The modern oral hypoglycemic agents produce undesirable and side effects. Thus, alternative therapy is required; a need of hour is to shift towards the different indigenous plant and herbal formulations (Satyanarayana et al., 2006). Recently, some medicinal plants have been

reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and anti-hyperlipidemic remedies. Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. Anti-hyperglycemic effects of these plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Along with the synthetic drugs, many agents of the plant origin are also in use particularly for the treatment of non-insulin dependent diabetes mellitus (NIDDM). More than 400 plant species having hypoglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having antidiabetic effect. (Neelesh et al., 2010)

The antidiabetic activity of herbs depends upon variety of mechanisms. The mechanism of action of herbal anti-diabetic could be grouped as- adrenomimeticism, pancreatic beta cell potassium channel blocking, cAMP (2nd messenger) stimulation (Marles and Farnsworth, 1996), inhibition in renal glucose reabsorption (Tahraoui et al., 2007), stimulation of insulin secretion from beta cells of islets or/and inhibition of insulin degradative processes, reduction in insulin resistance (Pulok et al., 2006), providing certain necessary elements like calcium, zinc, magnesium, manganese and copper for the beta-cells, regenerating and/or repairing pancreatic beta cells, increasing the size and number of cells in the islets of Langerhans (Mohamed et al., 2006), stimulation of insulin secretion (Esmaeili et al., 2004), stimulation of glycogenesis and hepatic glycolysis (Miura et al., 2001), protective effect on the destruction of the beta cells (Kim et al., 2003), improvement in digestion along with reduction in blood sugar and urea (Krishnan, 1968), prevention of pathological conversion of starch to glucose (Sepha and, Bose, 1956), inhibition of β -galactocidase and α -glucocidase (Sharma and Mujumdar, 1990), cortisol lowering activities (Gholap and Kar, 2004), inhibition of α -amylase (Heidari et al., 2005) and preventing oxidative stress that is possibly involved in pancreatic β -cell dysfunction found in diabetes (Hideaki et al., 2005). Hence, the wide range of plant constituents could have different sites of action within the body, herbs exerts different mechanism of actions including the mechanism of actions of synthetic oral hypoglycemic drugs.

Chapter Three

PLANT PREVIEW

PLANT PREVIEW

3.1. *Achyranthes aspera* (Leaves)

A coarse, rambling or erect, distantly branched annual herb, 0.5 to 2 m high. Leaves are oblong-ovate to elliptic or obovate, 6 to 15 cm long, pointed at both ends, more or less hairy, though often nearly smooth. Flowers are green and about 5 cm in length. Sepals 4 or 5, filaments connate at the base, the stamens and staminodes square toothed or fimbriate, pale purplish. Ovary oblong, 1-ovuled; flowers unisexual. The spikes are rigid, elongated and 10 to 15 cm long. Buds point upwards but when the flowers open, they spread out from the sides. Seeds oblong, brown and 2 to 3 mm long. Fruits utricles, oblong or ovoid, indehiscent (Figure 1).

Common name: Bangla - Apang, Prickly Chaff Flower, Chaff-flower, Crocus stuff, Crokars staff, Hindi-Chirchita.

Scientific classification :

Kingdom : Plantae
Order : Caryophyllales
Family : Amaranthaceae
Genus : *Achyranthes*
Species : *A. aspera*

(Chopra et al., 1956; Husain et al., 1992; Handa et al., 2006; Wiart, 2006; Khare, 2007).

Medicinal Uses:

Used to treat cold with fever, heat stroke with headache, malaria, dysentery sometimes urinary tract lithiasis, chronic nephritis and edema. It is also used for the treatment of diabetes and Rheumatic arthritis (joint pain). In Ayurveda, used for treating vomiting, bronchitis, heart problems, hemorrhoids, itching, abdominal pains, ascites, etc. In India, used as abortifacient, decoction of leaves used in treatment of diarrhea and dysentery. Poultice of leaves used for rabies, hysteria, insect and snake bites (Akhtar and Iqbal, 1991; Bashir et al., 1992; Siddiqui and Hussain, 1992; Singh et al., 1996, Gokhale et al., 2002 and Vasudeva et al., 2006).

3.2. *Adhatoda zeylanica* (Leaves)

An evergreen, gregarious, stiff, perennial, shrub, 1.2-6.0 m in height. Leaves elliptic-lanceolate or ovate lanceolate, entire, 5-30 cm long, hairy, light green above, dark below, leathery; flowers large, white with red- or yellow-barred throats, in spikes with large bracts; capsules clavate, longitudinally channeled, 1.9-2.2 cm x 0.8 cm; seeds globular (Figure 2).

Common name: Bangla - Basok, Hindi-vasaka

Scientific classification:

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Lamiales

Family : Acanthaceae

Genus : *Adhatoda*

Species : *A. zeylanica*

(Chopra et al., 1956; Husain et al., 1992; Handa et al., 2006; Wiart, 2006; Khare, 2007).

Medicinal uses: Antidiabetic, antioxidant and antibacterial activity to its wide range of pharmacological activities which includes antiulcer, hepatoprotective, anti-inflammatory, antitussive, bronchodilator, antitubercular and radioprotective activities (Okunade et al., 2004; Bhattacharyya et al., 2005; Kumar et al., 2005; Shrivastava et al., 2006 and Karthikeyan et al., 2009).

3.3. *Alpinia nigra* (Rhizome)

A. nigra is a perennial rhizomatous herb, leaves sessile or very shortly petiolate, up to 50 x 15 cm, linear-lanceolate, acuminate, some pubescence on either side of the midrib below, otherwise glabrous; ligule c. 0.5 cm long, rounded, entire, pubescent; sheaths glabrous. Inflorescence paniculate, pubescent, usually rather lax with remote cincinni. Bracts membranous, up to 0.5 cm long (those at the base often much larger), each subtending a cincinnus of up to 4 shortly pedicellate flowers. Bracteoles tubular, membranous, splitting to the base readily (Figure 3).

Common name: Bangla - Jangli ada

Scientific classification :

Kingdom : Plantae
Class : Liliopsida
Order : Zingiberales
Family : Zingiberaceae
Genus : *Alpinia*
Species : *A. nigra*

(Chopra et al., 1956; Husain et al., 1992; Handa et al., 2006; Wiart, 2006; Khare, 2007).

Medicinal uses: Anti-diabetic, expectorant, carminative, rheumatism, sores, dyspepsia, bronchitis, kidney disease (Ojala, et al., 2000; Joy et al., 2001 and Alzoreky et al., 2003).

3.4. *Asparagus racemosus* (Root)

A. racemosus is a creeper, 1 to 2 meters tall, that is common throughout India and the Himalayas. It prefers to take root in gravelly, rocky soils. Has odd little pine-needle-like leaves that are uniform, and shiny green. In July it has minute, white flowers on short, spiky stems, and in September fruits turn into blackish-purple, globular berries (Figure 4).

Common name: Bangla - Shatomul, Wild Asparagus, Asparagus Root, Satavari.

Scientific classification :

Kingdom : Plantae
Order : Asparagales
Family : Asparagaceae
Genus : *Asparagus*
Species : *A. racemosus*

(Chopra et al., 1956; Husain et al., 1992; Handa et al., 2006; Wiart, 2006; Khare, 2007).

Medicinal uses: The roots are used in Ayurvedic medicine, following a regimen of processing and drying, with the name of Shatavari. It is used to treat the diabetes. It is also used as an anodyne, aphrodisiac and galactagogue. Shatavari is considered to be the main Ayurvedic rejuvenating female tonic for overall health and vitality. The reputed adaptogenic effects of Shatavari may be attributed to its concentrations of saponins, known as Shatavarins (Nanal et al., 1974; Dalvi et al., 1990; Kamat et al., 2000; Mandal et al., 2000; Goyal et al., 2003; Cornwell et al., 2004; Fukusaki and Kobayashi 2005).

3.5. *Bacopa monnieri* (Floating leaves)

B. monnieri is a perennial, creeping herb whose habitat includes wetlands and muddy shores. The leaves of this plant are succulent and relatively thick. Leaves are oblanceolate and are arranged oppositely on the stem. The flowers are small and white, with four or five petals. Its ability to grow in water makes it a popular aquarium plant. It can even grow in slightly brackish conditions. Propagation is often achieved through cuttings (Figure 5).

Common name: Bangla - Brahmi, Coastal Waterhyssop, Thyme-leafed gratiola

Scientific classification :

Kingdom : Plantae
Order : Lamiales
Family : Scrophulariaceae
Genus : *Bacopa*
Species : *B. monnieri*

(Chopra et al., 1956; Husain et al., 1992; Handa et al., 2006; Wiart, 2006; Khare, 2007).

Medicinal uses:

This plant has a number of uses in Ayurveda. It is a traditional treatment for epilepsy and asthma. It has antioxidant properties, reducing oxidation of fats in the bloodstream. However, anti-epilepsy properties seem to be in very high toxic and near lethal doses, so it's only used—at much lower non-toxic dosage—as an additive to regular epilepsy medication. Studies in humans show that an extract of the plant has antianxiety effects. It's floating leaf is used to treat the diabetes for the diabetic patient. It is listed as a nootropic, a drug that enhances cognitive ability. Laboratory studies on rats indicate that extracts of the plant improve memory capacity and motor learning ability. Recent studies suggest *Bacopa monnieri* may improve intellectual activity. The sulfhydryl and polyphenol components of *Bacopa monnieri* extract have also been shown to impact the oxidative stress cascade by scavenging reactive oxygen species, inhibiting lipoyxygenase activity and reducing divalent metals. It is used in Rebirthing therapy to accelerate trauma release and make continuous breathing easier. *Bacopa monnieri* is well known for its tranquilizing, sedative, cognitive enhancing, hepatoprotective and antioxidant action (Schaeffer et al., 2001; Carson and Riley, 2003; Guarner and Malagelada, 2003; Akunpelu and Onakoya, 2006; Sampathkumar et al., 2008; Ayyapan et al., 2010; Mathur et al., 2010).

3.6. *Coccinia indica* (Leaves)

It is an annual creeper which is found spreading on ground and twilling around the trees and supports around it. The stem is pentagonal in shape. Leaves are triangular or pentagonal in shape; it is dentate and has a length of 2 to 5 inch and breath of 2 inch. Flowers are monocieus and are bid in size. They are placed in bunches having about 2 to 4 flowers. These are of white color. Fruit id slimy in touch, pulpy and is barrel shaped. It is 1 to 2 inch in length. it is green in color when it is raw form which turns to scarlet red color when it ripes. It also possesses about ten white stripes on its posterior portion. This fruit posses numerous seeds inside. Its roots are long and resemble tuberous roots. According to ayurveda there are two types of varieties (Figure 6).

Common Name: Telakucha in Bangla, Hindi name- kundru, Sanskrit name- imbi /tundika, Gujarati name – tindora.

Scientific classification :

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Cucurbitales

Family : Cucurbitaceae

Genus : *Coccinia*

Species : *C. indica*

(Chopra et al., 1956; Husain et al., 1992; Handa et al., 2006, Wiart, 2006; Khare, 2007).

Medicinal uses:

It is a good wound healer and reduces any kind of inflammation occurring in body. It is good appetizer and helps in improving digestion. It is a good laxative and stimulates liver for proper secretion of bile juices. It is also used in wormal infestation especially in amoebiasis caused by entamoeba hystolitica. It also purifies blood. It is also helpful in expelling the extra amount of mucus accumulated in the respiratory tract. It also controls the frequency of micturation and widely used in diabetes as it controls the glucose level in the blood. It also helps in opening the pores in the skin so as to facilitate the easy secretion of sweat thus expelling out the toxin in the body (Anjaria et al., 2002; Venkateshwaran and Pari, 2003; Akhtar et al., 2007; Umbreen et al., 2008; Shaheen et al., 2009; Baghel et al., 2011).

3.7. *Ficus racemosa* (Bark)

F. racemosa, a member of the fig family, is an evergreen tropical fig tree native to Asia and Australia. It is sometimes used as a bonsai. This tree has edible fruit and works well as a shade tree. There are some characteristics that all *F. racemosa* trees have in common. *F. racemosa* can grow over 40 feet tall and 20 to 40 feet wide. The oval leaves measure 3 to 4 inches long and are dark green. The fruit is 3/4 inch to 2 inches long, circular and grows directly on the trunk (Figure 7).

Common name: Bangla – Jogyadumur, Cluster fig, country fig, redwood fig, Indian fig.

Scientific classification :

Kingdom	:Plantae
Division	:Magnoliophyta
Class	:Magnoliopsida
Order	:Rosales
Family	:Moraceae
Genus	: <i>Ficus</i>
Species	: <i>F. racemosa</i>

(Chopra et al., 1956; Husain et al., 1992; Handa et al., 2006, Wiart, 2006; Khare, 2007)

Medicinal uses:

The roots, bark-skin, fruits, latex and leaves of udumbara have great medicinal value. Udumbara is used both, internally as well externally, externally; the latex is applied on chronic infected wounds to alleviate edema, pain and to promote the healing. The tender leaf buds are applied on the skin, in the form of paste, to improve the complexion; the decoction of leaves is salutary in washing the wounds for better cleansing and healing. The decoction of its bark-skin is an effective gargle in stomatitis and sore throat. Application of latex alleviates the edema in adenitis, parotitis, orchitis, traumatic swelling and toothache. In diabetes, the ripe fruits or bark-skin decoction is beneficial, as it works well as anti-diuretic. The decoction of leaves is an effective remedy in glandular swelling, abscess, chronic wounds, cervical adenitis etc. In uterine bleeding, abortion, leucorrhea and vaginal bleeding the decoction of its bark-skin is given orally, as well as in a form of basti (enema). The latex mixed with sugar is benevolent in sexual debility in males (Hemaiswarya et al., 2009; Singh and Goel, 2009; Uma et al., 2009; Ahuja et al., 2011; Patil et al., 2011; Sawarkar et al., 2011).

3.8. *Gymnema sylvestre* (Bark)

G. sylvestre is a herb native to the tropical forests of southern and central India where it has been used as a naturopathic treatment for diabetes for nearly two millennia. Large climbers, rooting at nodes, leaves elliptic, acuminate, base acute to acuminate, glabrous above sparsely or densely tomentose beneath; Flowers small, in axillary and lateral umbel like cymes, pedicels long (Figure 8).

Common name: Bangla – Gurmur, Hindi: Gurmar, Tamil - Sirukurinchaan

Scientific classification :

Kingdom : Plantae

Order : Gentianales

Family : Asclepiadaceae

Genus : *Gymnema*

Species : *G. sylvestre*

(Chopra et al., 1956; Husain et al., 1992; Handa et al., 2006; Wiart, 2006; Khare, 2007).

Medicinal uses:

The herb has been shown to reduce blood sugar levels when used for an extended period of time. Additionally, *Gymnema* reduces the taste of sugar when it is placed in the mouth, thus some use it to fight sugar cravings. From extract of the leaves were isolated glycosides known as Gymnemic acids, which exhibit anti-sweet activity. Extracts of *Gymnema* is not only claimed to curb sweet tooth but also for treatment of as varied problems as hyperglycemia, obesity, high cholesterol levels, anemia and digestion. According to the Sushruta of the Ayurveda it helps to treat Madhumeha ie glycosuria (Bishayee and Chatterjee, 1994; Kalidass and Mohan, 2010 ; Saneja et al., 2010).

3.9. *Momordica chinensis* (Fruit)

Momordica is a genus of about 80 species of annual or perennial climbers herbaceous or rarely small shrubs belonging to the family Cucurbitaceae, natives of tropical and subtropical Africa and Asia and Australia. Most species produce floral oils and are visited by specialist pollinators in the apid tribe Ctenoplectrini. Some *Momordica* species are grown in cultivation for their fleshy fruit, which are oblong to cylindrical in shape, orange to red in colour, prickly or warted externally, and in *M. charantia* burst when ripe, generally with elastic force, into irregular valves (Figure 9).

Common name: Bangla –Karollia, Balsam apple, balsam pear, bitter cucumber

Scientific classification :

Kingdom	:Plantae
Division	:Magnoliophyta
Class	:Magnoliopsida
Order	:Cucurbitales
Family	:Cucurbitaceae
Genus	: <i>Momordica</i>
Species	: <i>M. chinensis</i>

(Chopra et al., 1956; Husain et al., 1992; Handa et al., 2006; Wiart, 2006; Khare, 2007).

Medicinal uses:

Fruit, seeds, seed oil, leaves; uses: anti-diabetic, anti-infective, antipyretic, anti-helminthic, laxative, possible anti-fungal, androgenic, antiviral, anti-malarial actions; possibly useful for infertility; precautions: pregnancy, lactation, children, patients taking hypoglycemic medications; may cause uterine bleeding or contractions, hepatotoxicity; seeds are toxic to children (Ahmad et al., 1999; Raza et al., 2000; Alqasumi et al., 2009; Rahmatullah et al., 2010).

3.10. *Swertia chirata* (Whole part)

The plant can be grown in a variety of soils with sandy loam rich in carbon and humus. It is also found in open ground and recently slash-and-burnt forests *S. chirata* has an erect, about 2–3 ft long stem, the middle portion is round, while the upper is four-angled, with a prominent decurrent line at each angle. The stems are orange brown or purplish in colour, and contain large continuous yellowish pith. The root is simple, tapering and stout, short, almost 7 cm long and usually half an inch thick, flowering in *S. chirata* is in the form of numerous small, axillary, opposite, lax cymes arranged as short branches and the whole inflorescence is 2 ft long. Flowers are small, stalked, green-yellow, tinged with purple colour, rotate and tetramerous. The upper surface of the petal has a pair of nectaries covered with oblong scales and ending as fringes. Fruit is a small, one-celled capsule with a transparent yellowish pericarp. It dehisces from above, septicidally into two valves. Seeds are numerous, minute, many-sided and angular (Figure 10).

Common name: Bangla – Chirata, Hindi - Chirayata, Bhunimba, Bhuchiretta, Charayatah, Chiretta, Chiraita, Indian Gentian, Kiriayattu, Kiryat-charayatah, Mahatita, Nila-vemu, Nila-vembu, Arabic - Qasabuz-Zarirah.

Scientific classification :

Kingdom : Plantae
Order : Gentianales
Family : Gentianaceae
Genus : *Swertia*
Species : *S. chirata*

(Chopra et al., 1956; Husain et al., 1992; Handa et al., 2006; Wiart, 2006; Khare, 2007).

Medicinal uses:

It is an excellent medicine for strengthening the stomach and promoting its action thus used in treating stomach disorders like dyspepsia and diarrhoea. its anthelmintic properties help in destroying intestinal worms. An infusion of the herb is taken for this purpose. The root of the plant is useful in curing hiccups and vomiting. The herb can also be used for range of other diseases and conditions including leprosy, leucoderma, scabies, neuro-muscular disorders, menorrhagia, menstrual irregularity, urinary disease, heart disease, asthma, cough, dyscrasia, ulcer, jaundice and anaemia. It also reduces the blood glucose level (Joshi and Dhawan, 2005; Sultana et al., 2007; Kumar et al., 2010; Laxmi et al., 2011; Negi et al., 2011).

3.11. *Swietenia mahagoni* (Seed)

Swietenia is a genus of trees in the mahogany family Meliaceae. As a timber, both *S. macrophylla* and *S. mahagoni* are both grown in plantations in several Asian countries such as Fiji, Indonesia, India, and Bangladesh. It is usually taken to consist of three species, geographically separated. They are medium-sized to large trees growing to 20–45 m tall, and up to 2 m trunk diameter. The leaves are 10–30 cm long, pinnate, with 3-6 pairs of leaflets, the terminal leaflet absent; each leaflet is 5–15 cm long. The leaves are deciduous to semi-evergreen, falling shortly before the new foliage grows. The flowers are produced in loose inflorescences, each flower small, with five white to greenish-yellowish petals. The fruit is a pear-shaped five-valved capsule 8–20 cm long, containing numerous winged seeds about 5–9 cm long (Figure 11).

Common name: Bangla – Mahagoni, Big Leaf Mahogany, broad-leaved Mahogany, Brazilian Mahogany, Honduras Mahogany, Large-leaved Mahogany, genuine mahogany, Sky Fruit, Tropical American Mahogany.

Scientific classification :

Kingdom : Plantae

Order : Sapindales

Family : Meliaceae

Genus : *Swietenia*

Species : *S. mahagonee*

(Chopra et al., 1956; Husain et al., 1992; Handa et al., 2006; Wiart, 2006; Khare, 2007).

Medicinal uses:

The fruits of *S. mahagoni* are called "sky fruit", because they seem to hang upwards from the tree. The "sky fruit" concentrate is sold as a natural remedy that is said to improve blood circulation, skin and reduce blood glucose level. It is also said to have Viagra-like qualities regarding erectile dysfunction. Consumption of the sky fruit concentrate is approved by Malaysia Ministry of Health (Matsuse et al., 1998; Ahmad and Beg, 2001; Majid et al., 2001; Sahgal et al., 2009; Sharma et al., 2011).

3.12. *Syzygium cumini* (Seed)

The evergreen Jam plant is originally from Indonesia and India; it now grows abundantly in Suriname. It has dispersed spontaneously in the wild as the seeds are sought by birds. An evergreen tropical tree 50 to 100 ft. tall, with oblong opposite leaves that are smooth, glossy; they have a turpentine smell. The bark is scaly gray and the trunk forks into multiple trunks about 3 - 5' from the ground. Jamun has fragrant white flowers in branched clusters at stem tips and purplish-black oval edible berries. The juicy fruit-pulp contains resin, gallic acid and tannin; it tastes usually from acid to fairly sweet. The somewhat astringent, jamun fruit can be utilized for juice (Figure 12).

Common name: Bangla - Kalo Jam, Java plum, jambul, jamun, jaman, black plum, faux pistachier, Indian blackberry, jambol, doowet, jambolan.

Scientific classification :

Kingdom : Plantae

Order : Myrtales

Family : Myrtaceae

Genus : *Syzygium*

Species : *S. cumini*

(Chopra et al., 1956; Husain et al., 1992; Handa et al., 2006; Wiart, 2006; Khare, 2007).

Medicinal uses:

All parts of the java plum can be used medicinally and it has a long tradition in alternative medicine. The bark has anti-inflammatory activity and is used In India for anemia, the bark and seed for diabetes which reduce the blood sugar level quickly, the fruit for dysentery and leave's juice for gingivitis (bleeding gums). In the Philippines and Suriname wine is made from the fermented fruit (Bhandary et al., 1995; Chhetri et al., 2005; Jain et al., 2005; Nagaraju et al., 2006; Udayan et al., 2006; Albuquerque et al., 2007; Ayyanar, 2008; Ayyanar and Subash-Babu, 2012).

3.13. *Trigonella foenum-graecum* (Seed)

An erect annual to 50 cm. which may be branched, the leaves are trifoliate and the leaflets oblong-lanceolate, to 5 cm. Its yellowish flowers 12 – 18 mm long (1 – 2) are in the leaf axils. The fruits are almost straight and flattened with a pronounced beak; they are 50 – 110 mm long excluding the beak of 10 – 35 mm. The seeds are brownish, about 1/8 inch long, oblong, rhomboidal, with a deep furrow dividing them into two unequal lobes; they are contained, ten to twenty together, in long, narrow, sickle-like pods (Figure 13).

Common names: Bangla, India – Methi, Fenugreek; fenugrec (France), fieno greco (Italy)

Scientific classification :

Kingdom	: Plantae
Division	: Magnoliophyta
Class	: Magnoliopsida
Order	: Fabales
Family	: Fabaceae
Genus	: <i>Trigonella</i>
Species	: <i>T. foenum-graecum</i>

(Chopra et al., 1956; Husain et al., 1992; Handa et al., 2006, Wiart, 2006; Khare, 2007)

Medicinal uses:

Abdominal colic, bronchitis, cough, sprains, treatment after childbirth, digestive, abortive, purgative, galactagogue, diabetes, emmenagogue, stomachic, anthelmintic, asthma, emphysema, aphrodisiac, reconstituent, sedative for palpitations, icterus, gastro-intestinal troubles, fevers, constipation, sterility (Randhir et al., 2004; Pande et al., 2011 and Sundaram and Purwar, 2011).



Figure 1: *Achyranthes aspera*

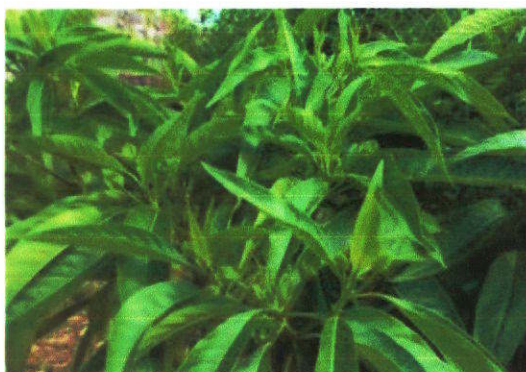


Figure 2: *Adhatoda zeylanica*



Figure 3: *Alpinia nigra*



Figure 4: *Asparagus racemosus*



Figure 5: *Bacopa monnieri*

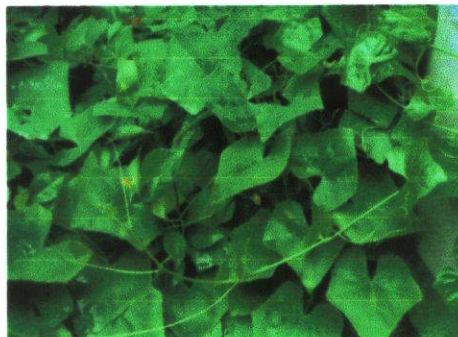


Figure 6: *Coccinia indica*

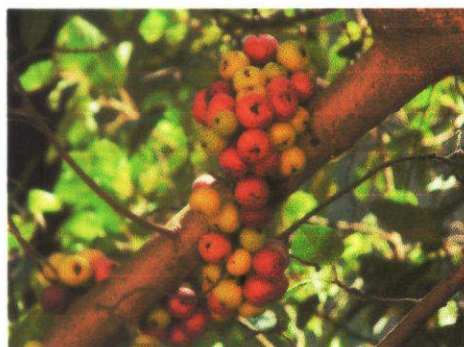


Figure 7: *Ficus racemosa*



Figure 8: *Gymnema sylvestre*



Figure 10: *Swertia chirata*



Figure 9: *Momordica chinensis*



Figure 11: *Swetania mahagonee*



Figure 12: *Syzygium cumini*

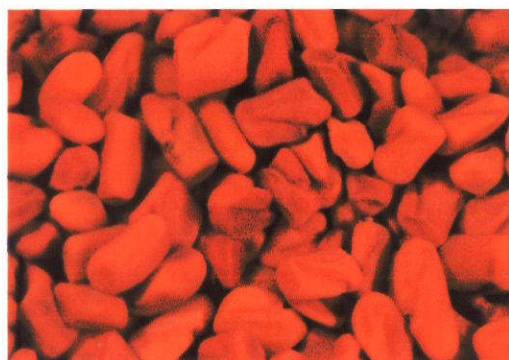


Figure 13: *Trigonella foenum-graecum*

Chapter Four

MATERIALS AND METHODS

MATERIALS

4.1 Instruments used in the experiments

1. Laminar air flow (OptiMair; ACB-4A, ESCO Technologies Inc. Singapore)
2. Incubator (WTB binder, Titling, Germany)
3. Autoclave
4. Spectrophotometer (Hitachi, U-2910)
5. Homogenizer (WiseTis, HG-15D)
6. pH meter (pH Tester H196107, Hanna Instruments, USA)
7. Water bath (Thermostatic water bath, Shanghai, China)
8. Digital Balance (OHAUS, PA104 Corp. USA)
9. Water Distiller
10. Refrigerator
11. Micropipette (Gilson, France)
12. Beaker
13. Test tubes
14. Petri dish
15. Inoculating loop (Nicrome, 2mm), etc.

4.2 Chemical used in the experiment

1. Methanol (Merck, Germany)
2. Hexen (Merck, Germany)
3. Diethyl Ether (Merck, Germany)
4. Ethyl Acetate (Merck, Germany)
5. Chloroform (Merck, Germany)
6. Di Methyl Sulphoxide (DMSO) (Merck, Germany)
7. Nutrient Broth (HIMEDIA, M002)
8. Agar powder (HIMEDIA, M002)

4.3 Plant Material

4.3.1 Collection of plant samples

The medicinal plants were collected from different parts of Khulna, Satkhira, Narail and Jessore district (Table 1).

4.3.2. Preparation of Methanol extract

Used parts of the collected plants were washed by tap-water to remove undesirable materials. After making desirable size and shape by means of cutting with a sharp blade the plant materials were shed dried until complete evaporation of water and the dried materials were ground to coarse powder using a mechanical grinder. Twenty five grams (25 g) of powder of each sample was taken into 100 mL of 100 % methanol and after homogenization kept at dark in air tight bottle at room temperature for 72 hours. The extract was then filtered by Whatman No. 1 filter paper followed by continuous air drying to get solid extract. The solid extract was weighted and kept in the refrigerator at 4 °C. Ten milligrams (10 mg) of the prepared extract was dissolved in 1 mL 100 % methanol (10 mg/mL) to conduct experiments.

4.3.3. Fractionation of samples

At first, 10 g of dried powder of each sample was soaked in 200 mL of 100 % hexen for 30 min with intermittent shaking. The plant extracts were filtered through Whatman No. 1 filter paper. The collected liquids were dried until a constant dry weight of each extract was obtained. Same procedure was repeated for the remaining dry plant residues using the other four solvents; diethyl ether, chloroform, ethyl acetate and methanol respectively (Figure 1). The dried extracts were measured and are shown in percentage of weight of the dry powder (Appendix: Table 2) and stored at 4° C. Twenty milligrams (20 mg) of prepared extract was dissolved in 1 mL 100 % DMSO (20 mg/mL) for experiment.

4.4. Bacterial strains and their maintenance

For screening antibacterial activity, six pathogenic bacteria (Table 3) were collected from ICDDR'B as clinical isolates and were maintained in nutrient broth (NB) media.

Table 1: Plant sample with scientific name, Bangla (Local) Name and used part

Scientific name	Bangla name	Used part
<i>Achyranthes aspera</i>	Apang	Leaf
<i>Adhatoda zeylanica</i>	Basok	Leaf
<i>Alpinia nigra</i>	Jongli Ada	Rhizome
<i>Asparagus racemosus</i>	Shatomull	Root
<i>Bacopa monniera</i>	Brahmi	Floating leaf
<i>Coccinia indica</i>	Telakucha	Leaf
<i>Ficus racemosa</i>	Jogyadumur	Bark
<i>Gymnema sylvestre</i>	Gurmur	Bark
<i>Memordica chinensis</i>	Bittermelon	Fruits
<i>Swertia chirata</i>	Chirata	Whole part
<i>Swetania mahagonee</i>	Mehogonee	Seed
<i>Syzygium cumini</i>	Black Berry	Seed
<i>Trigonella foenum- graecum</i>	Methi	Seed

Source: Handa et al., 2006; Wiart, 2006; Khare, 2007

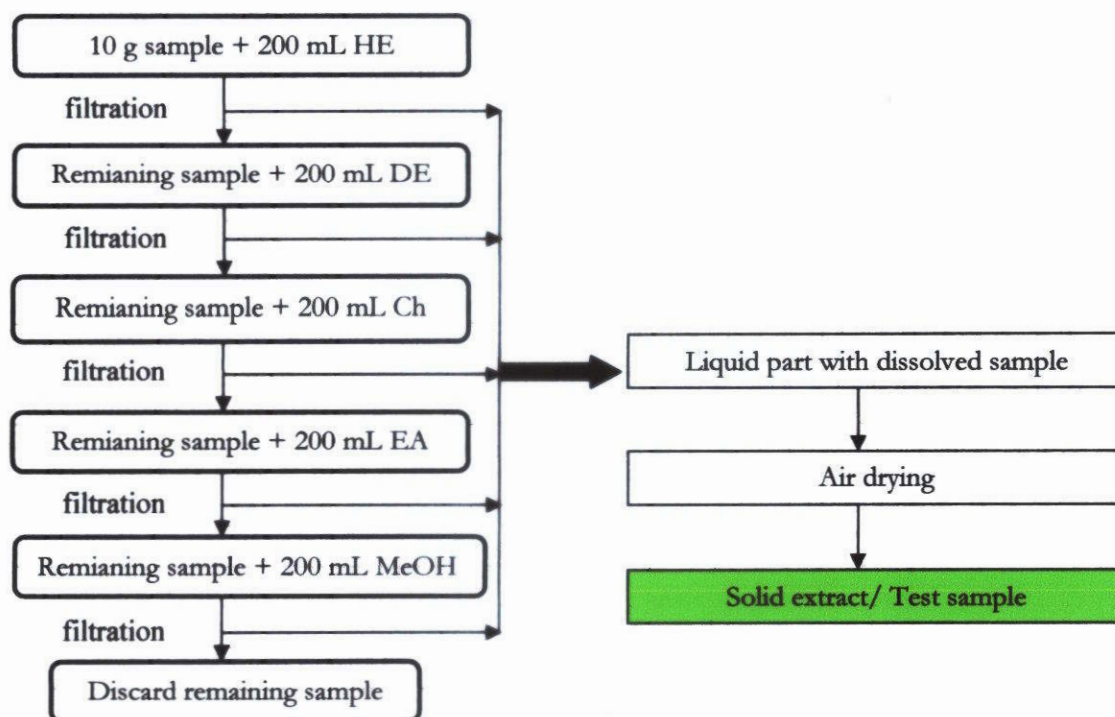


Figure 14: Schematic presentation of fraction of samples with five different solvents (HE- Hexen, DE- Diethyl Ether, EA- Ethyl Acetate, Ch- Chloroform, MeOH- Methanol)

Table 3: List of bacteria with diseases caused by them

Gram positive/negative	Bacteria	Disease
Negative	<i>Escherichia coli</i>	Gastroenteritis, urinary tract infections, and neonatal meningitis
	<i>Salmonella paratyphi A</i>	Paratyphoid A
	<i>Salmonella typhi</i>	Typhoid
	<i>Shigella dysenteriae</i>	Bacillary dysentery
	<i>Vibrio cholerae</i>	Cholera
Positive	<i>Staphylococcus aureus</i>	Scaled skin syndrome

Source: Pharmaceutical Microbiology, Hugo and Russell, 6th ed. (1998)

METHODOLOGY

3.4. Preparation of inoculum

Stock cultures were maintained at 4° C on nutrient agar slant. Active cultures for experiments were prepared by transferring a loop-full of cells from the stock cultures to test tubes of Nutrient Broth (pH 7 ± 0.2) that were incubated without agitation for 24 hrs at 37° C.

3.5. Preparation of the discs

Sterilized blank paper discs (6 mm in diameter, Oxoid Ltd.) were impregnated separately with the solution of test extract (10 mg/mL) to get a final concentration of 400 µg/disc. The discs were placed in sterilized culture plates with a sterile needle and left for a period of time in an aseptic condition for the complete evaporation of the solvents. Control discs (MeOH or DMSO) were also prepared in order to determine whether remaining solvent (if any) in disc shows any antimicrobial activity. For the preparation of control disc equal volume of solvent without test extracts was used to soak the paper disc. Tetracyclin (Oxoid Ltd.) was used as standard antibiotic discs (30 µg/disc) for positive control.

3.6. Disc Diffusion Method for antibacterial activity

The disc diffusion method was used to screen the antibacterial activity. From the overnight culture plate, small portion of a fresh colony was transferred into vial containing nutrient broth and incubated at 37° C until the growth reached the log phase (4-6 hrs; 5×10^6 cfu/mL). The NA plates were prepared by pouring 20 mL of molten media into sterile petriplates and were allowed to solidify. Then plates were seeded properly by pouring the culture broth with a Pasteur pipette to make bacterial lawn. The excess culture broth was withdrawn from the plates and allowed to dry. Discs impregnated with extracts (MeOH or DMSO as solvent) were placed at proportionate distance from each other using a sterile needle. The plates were incubated overnight at 37° C and inhibition zones were measured in millimeter (mm) with a transparent scale and noted down.

3.7. Determination of Inhibitory Concentration 50 (IC₅₀)

In this method an increasing in concentration of the extract (dissolved in particular solvent) were added to different test tubes containing particular culture. Calculated amount of extract was added from the stock solution (20 mg/mL) to the sterilized screw-capped one drum vials each containing 1 mL of NB to obtain seven different concentrations (0.5, 1, 2, 4, 6, 8, 10 µg/mL respectively). Then 10 µl of freshly grown inoculum (5×10^6 cfu/mL) was added to

each vial. Additional vials were also included as control; media with inoculums; media with extract (1, 2 and 4 mg/mL) and media with of solvent and bacteria. The first control tubes were included to check the turbidity of media due to bacterial growth, the second was to check turbidity caused by extract and the third control tubes were to check whether the solvent itself caused any inhibition. All the tubes were incubated at 37° C. After incubation, ODs were taken by spectrophotometer (Hitachi, U-2910) at 700 nm, noted down and then calculated to find out the percentage of inhibition and finally IC₅₀ value for each extract.

3.8. Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of fractionated extracts was determined by the tube dilution method. In this method, an increasing in concentration of extract was added to different test tubes.

Sterilized screw-capped one drum vials each containing 2 mL of nutrient broth were taken. Then calculated amount of extract of different fractions was added from the stock solution (20 mg/mL) to the vials to obtain a range of different concentrations (0.2, 0.5, 1, 1.5, 2, 2.5, 3 and 4 mg/mL) of the extract. Then 10 µL of freshly grown inoculums (5×10^6 cfu/mL) was added to each vial. Additional vials were also included as control, designated as media with inoculums; media with 2 mL of extract and media and 50 µL of solvent and bacteria. All the tubes were incubated at 37° C. After incubation, vials having lowest concentrations of extract showing no turbidity were primarily selected as MIC of the extract. However, in case of excessively stained extract subculture from the tubes were made to determine the MIC. The whole process was replicated for testing each extract.

3.6 Determination of Minimum Bactericidal Concentration (MBC) of extracts

The minimum bactericidal concentration (MBC) of fractionated extracts was determined by measuring the viability loss of bacterial culture with an increasing in concentration of extracts. Vials containing different concentration of extracts were plated out on NA plate to test the viability. Null colony formation indicated the MBC concentration(s).

Chapter Five

RESULTS

5.4 Minimum Bactericidal Concentration (MBC)

MBC values were determined by plating suspensions from culture vials of MIC assay. The vials that showed no visual growth were streaked onto NA plate to check the viability and at a certain concentration no visible growth of bacteria was found onto the plate. Thus, MBC values of extracts were recorded (Appendix: Table 7) and streak plates are presented in figure 31, 32 and 33. At 4 mg/mL, *S. typhi*, *S. dysenteriae* and *S. aureus* were completely inhibited by MeOH fraction of *S. cumini*, while *S. paratyphi* A was absolutely inhibited at 3 mg/mL. *S. typhi* was entirely inhibited by both *G. sylvestre* and *S. chirata* at 2 mg/mL and which was equal to that of *C. indica* against *S. paratyphi* A.

Table 4: Mean diameter of zone of inhibition (mm $\pm$ SD)

Bacteria	Aa	Az	An	Ar	Bm	Ci	Fr	Gs	Mc	Swc	Sm	Sc	Tfg	MeOH	Tet.
<i>Salmonella typhi</i>	-	-	-	-	-	-	-	18 $\pm$ 0.71	-	14 $\pm$ 1.41	-	13 $\pm$ 0.71	-	NA	24.25 $\pm$ 0.35
<i>Salmonella paratyphi A</i>	-	-	-	8.5 $\pm$ 0	-	17.5 $\pm$ 0.71	-	-	-	10 $\pm$ 0	8.5 $\pm$ 0	11.5 $\pm$ 0.71	-	-	13 $\pm$ 0
<i>Escherichia coli</i>	7 $\pm$ 0	6.5 $\pm$ 0	7.5 $\pm$ 0	-	6.5 $\pm$ 0	7 $\pm$ 0	9 $\pm$ 0	6.75 $\pm$ 0.35	7.25 $\pm$ 0.35	7 $\pm$ 0	-	-	-	-	16 $\pm$ 0
<i>Vibrio cholerae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	19.75 $\pm$ 0.35
<i>Shigella dysenteriae</i>	-	-	6.5 $\pm$ 0	7.25 $\pm$ 0.35	-	-	8.5 $\pm$ 0	-	-	-	8 $\pm$ 1.41	14.75 $\pm$ 0.35	8.25 $\pm$ 1.06	-	8 $\pm$ 0
<i>Staphylococcus aureus</i>	-	-	7.75 $\pm$ 0.35	-	6.5 $\pm$ 0	6.75 $\pm$ 0.35	-	-	7 $\pm$ 0.71	-	-	8.75 $\pm$ 0.35	-	-	8.25 $\pm$ 0.35

Disc diameter: 6 mm, Concentration: 400 μ g/disc; Aa- *Achyranthes aspera*; Az- *Adhatoda zeylanica*; An- *Alpinia nigra*; Ar- *Asparagus racemosus*; Bm- *Bacopa monniera*; Ci- *Coccinia indica*; Fr- *Ficus racemosa*; Gs- *Gymnema sylvestre*; Mc- *Memordica chinensis*; Swc- *Swertia chirata*; Sm- *Swetania mahagonee*; Sc- *Syzygium cumini*; Tfg- *Trigonella foenum- graecum*; Tet- tetracyclin

Table 5: Mean diameter of zone of inhibition (mm $\pm$ SD) of fractionated extracts

Culture	Sample					
	ScH	ScDE	ScCH	ScEA	ScMeOH	DMSO
<i>S. typhi</i>	6.5 $\pm$ 0	-	-	8 $\pm$ 0	9.5 $\pm$ 0.71	-
<i>S. paratyphi</i> A	6.5 $\pm$ 0	-	7 $\pm$ 0	6.5 $\pm$ 0	8.25 $\pm$ 0.35	-
<i>Shigella dysenteriae</i>	7 $\pm$ 0	6.5 $\pm$ 0	6.5 $\pm$ 0	-	11.75 $\pm$ 0.35	-
<i>Staphylococcus aureus</i>	-	6.5 $\pm$ 0	-	-	8 $\pm$ 0	-

Culture	Sample					
	ChH	ChDE	ChCH	ChEA	ChMeOH	DMSO
<i>S. paratyphi</i> A	7 $\pm$ 0	-	-	-	8.5 $\pm$ 0	-
<i>S. typhi</i>	GsH	GsDE	GsCH	GsEA	GsMeOH	-
	-	6.5 $\pm$ 0	-	-	9.75 $\pm$ 0.35	-
	SwcH	SwcDE	SwcCH	SwcEA	SwcMeOH	-
	-	-	-	-	8.5 $\pm$ 0	-

Disc diameter: 6mm, Concentration: 400 μ g/disc; *Syzygium cumini* (Sc), *Coccinia indica* (Ci), *Gynemna sylvestre* (Gs), *Swertia chirata* (Swc). H- Hexen; DE- Diethyl Ether; CH- Chloroform; EA- Ethyl Acetate; MeOH- Methanol; DMSO- Di Methyl Sulphoxide

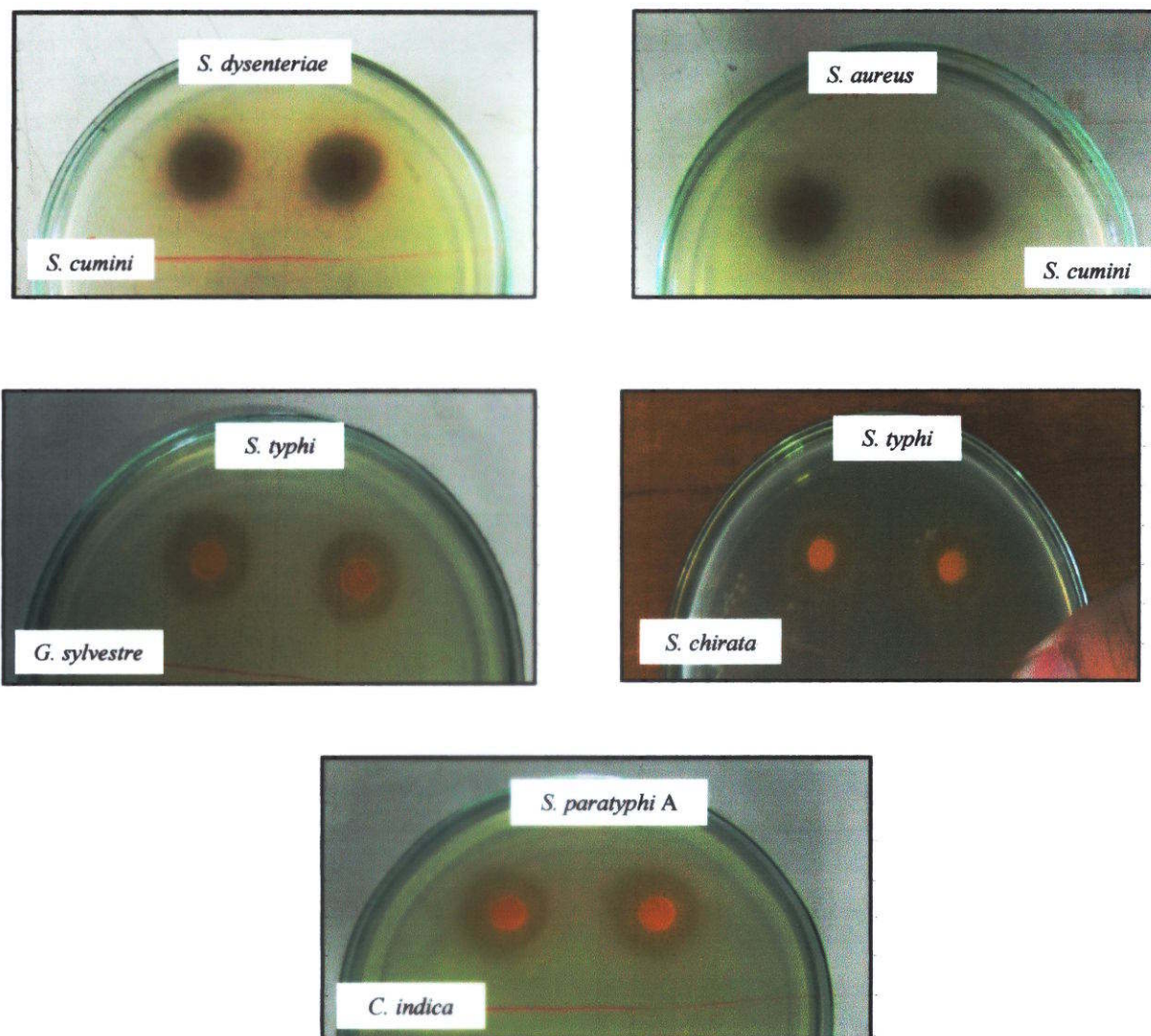


Figure 15: Zone of inhibition of methanol extracts of *C.indica*, *G. sylvestre*, *S. chirata* and *S. cumini* against test bacteria

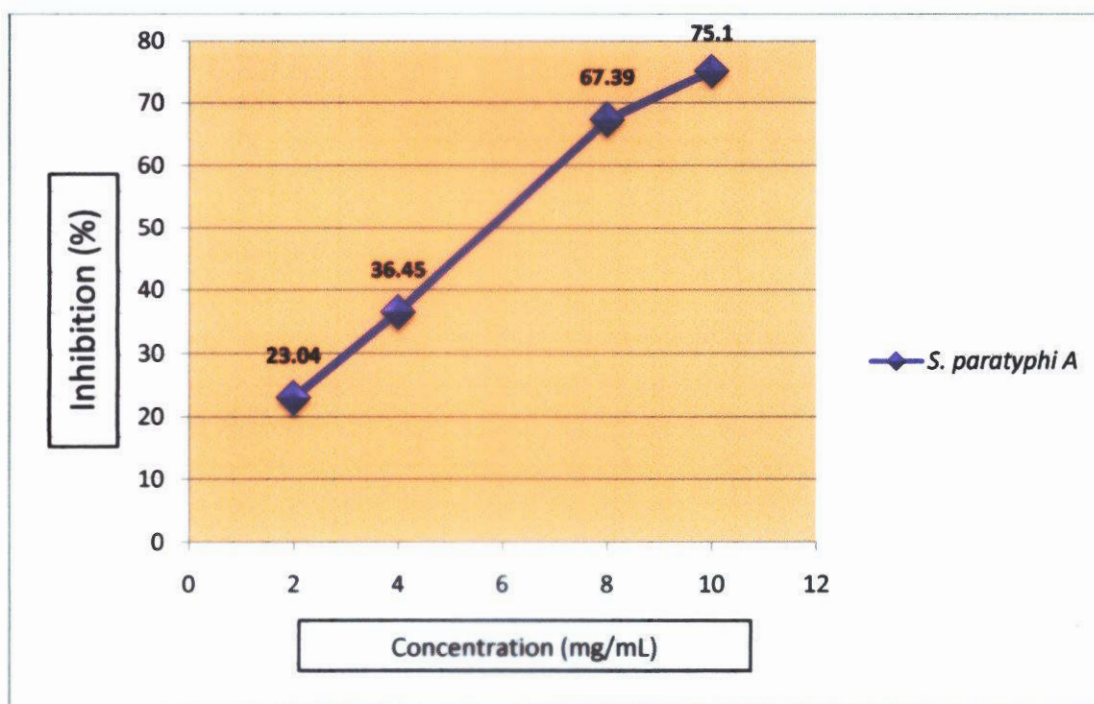


Figure 16: Dose dependent inhibition (%) of *S. paratyphi A* by the methanol extract of *C. indica*

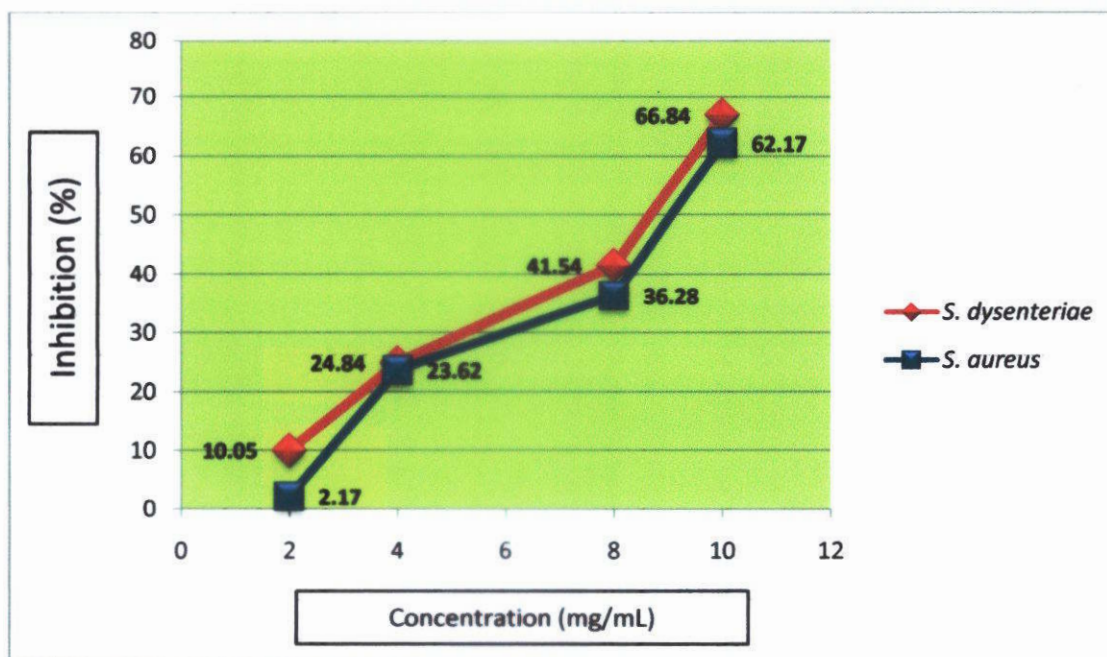


Figure 17: Dose dependent inhibition (%) of *S. dysenteriae* and *S. aureus* by the methanol extract of *S. cumini*

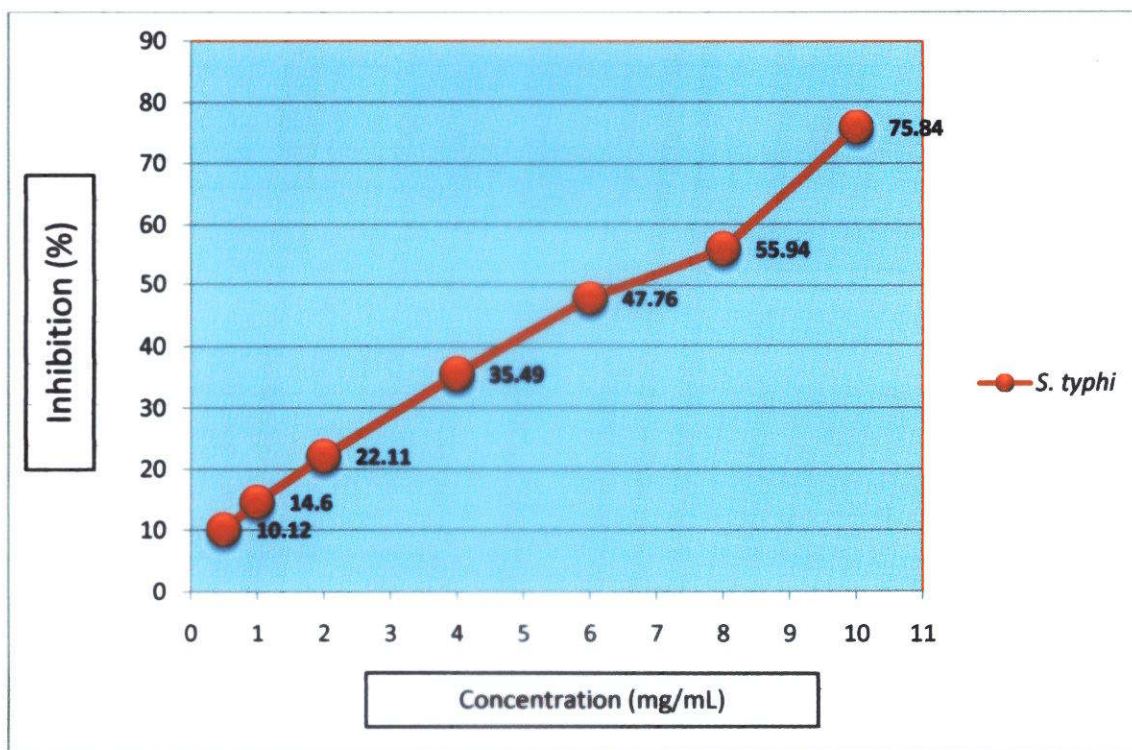


Figure 18: Dose dependent inhibition (%) of *S. typhi* by the methanol extract of *G. sylvestre*

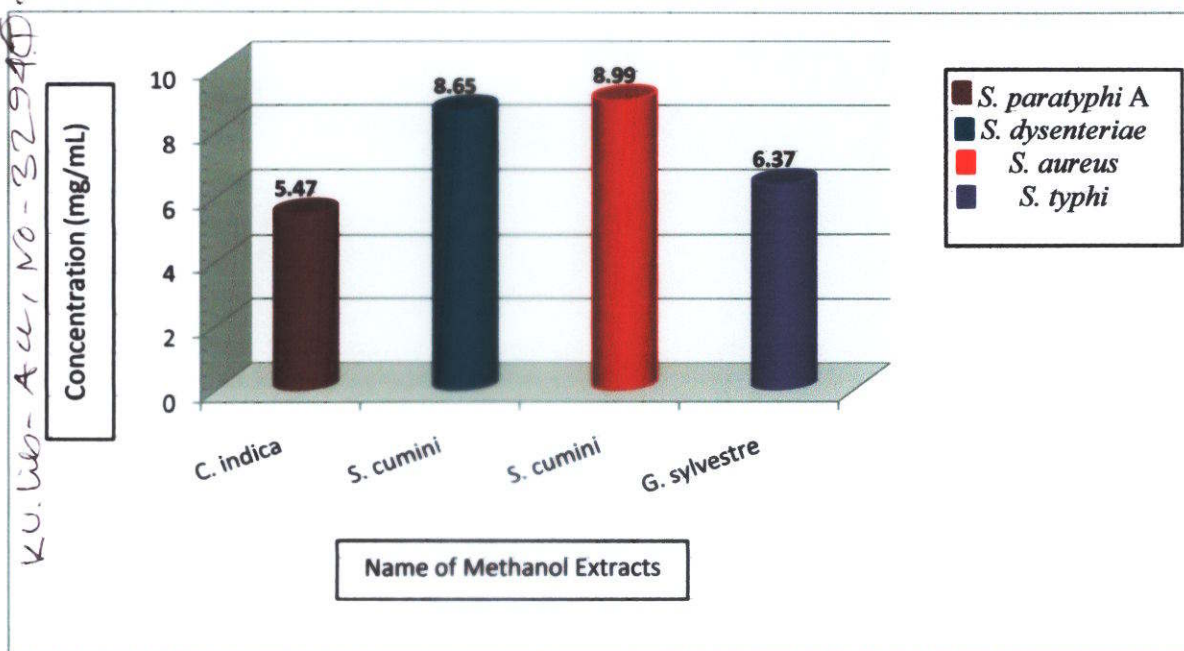


Figure 19: IC₅₀ values of three methanol extracts against four test bacteria

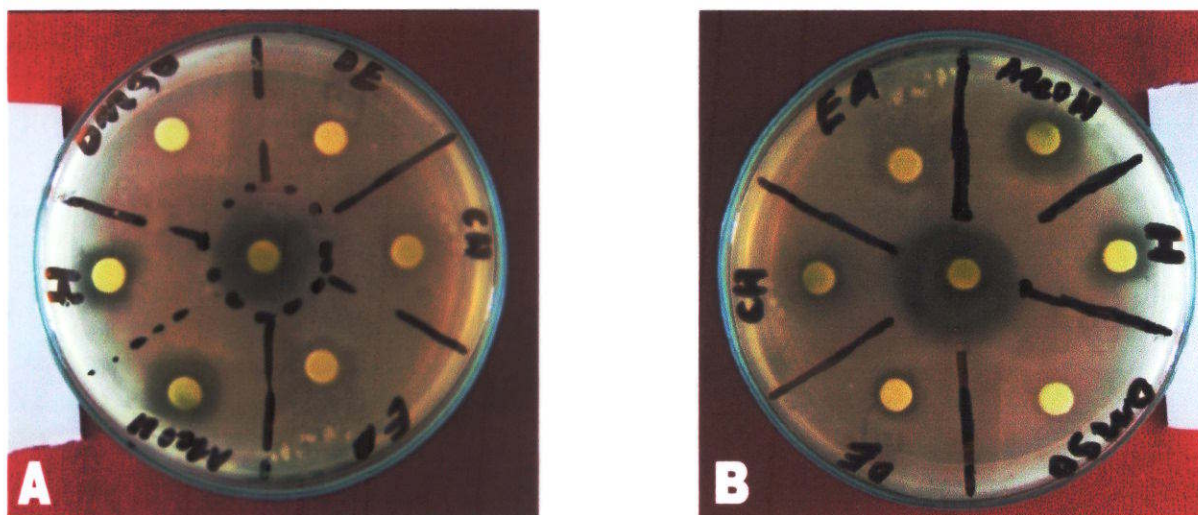


Figure 20: Zone of inhibition of fractionated extracts of *C. indica* (A) and *S. cumini* (B) against *S. paratyphi* A

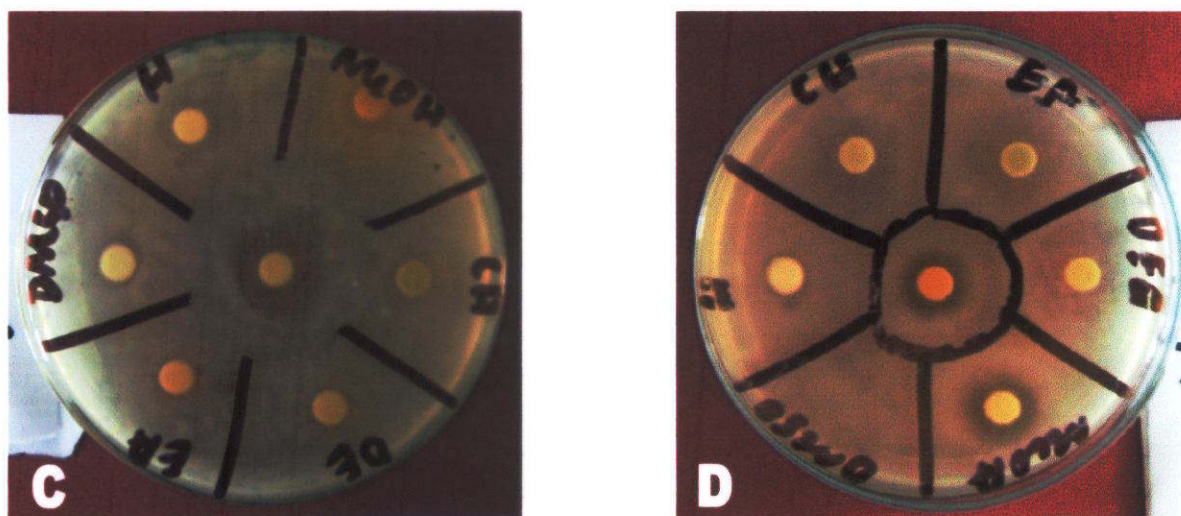


Figure 21: Zone of inhibition of fractionated extracts of *G. sylvestre* (C) and *S. chirata* (D) against *S. typhi*

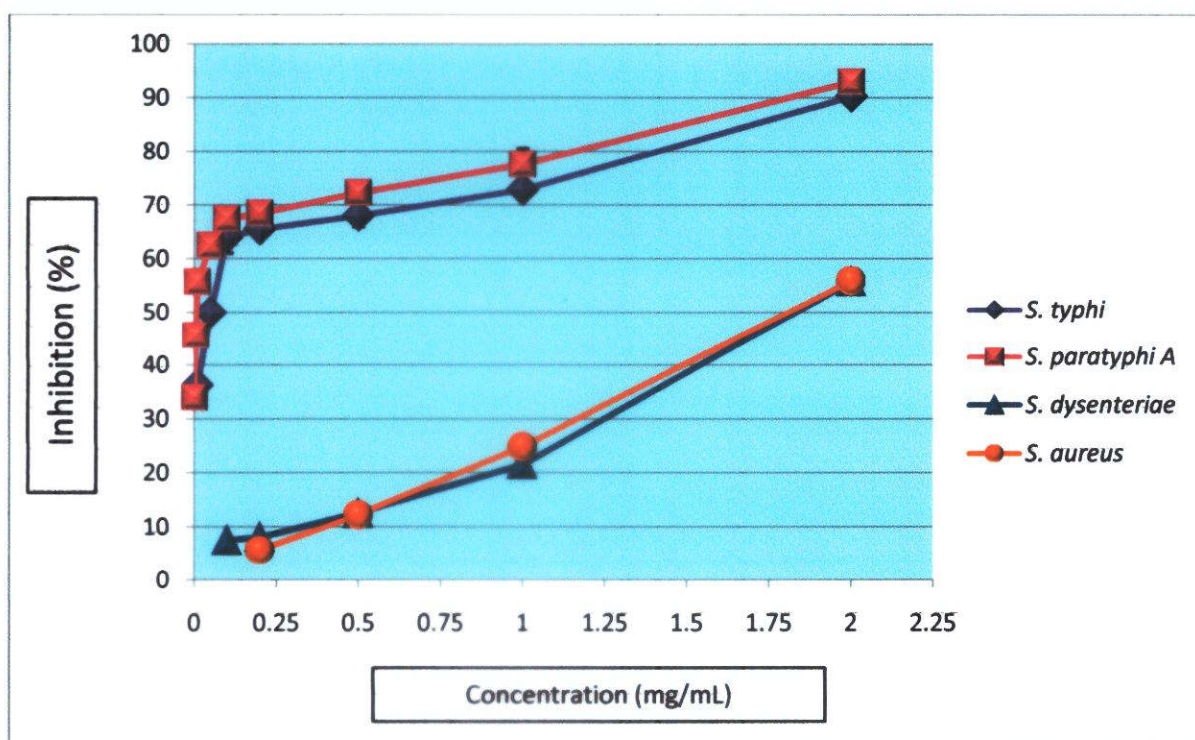


Figure 22: Dose dependent inhibition (%) of four test bacteria by MeOH fraction of *S. cumini*

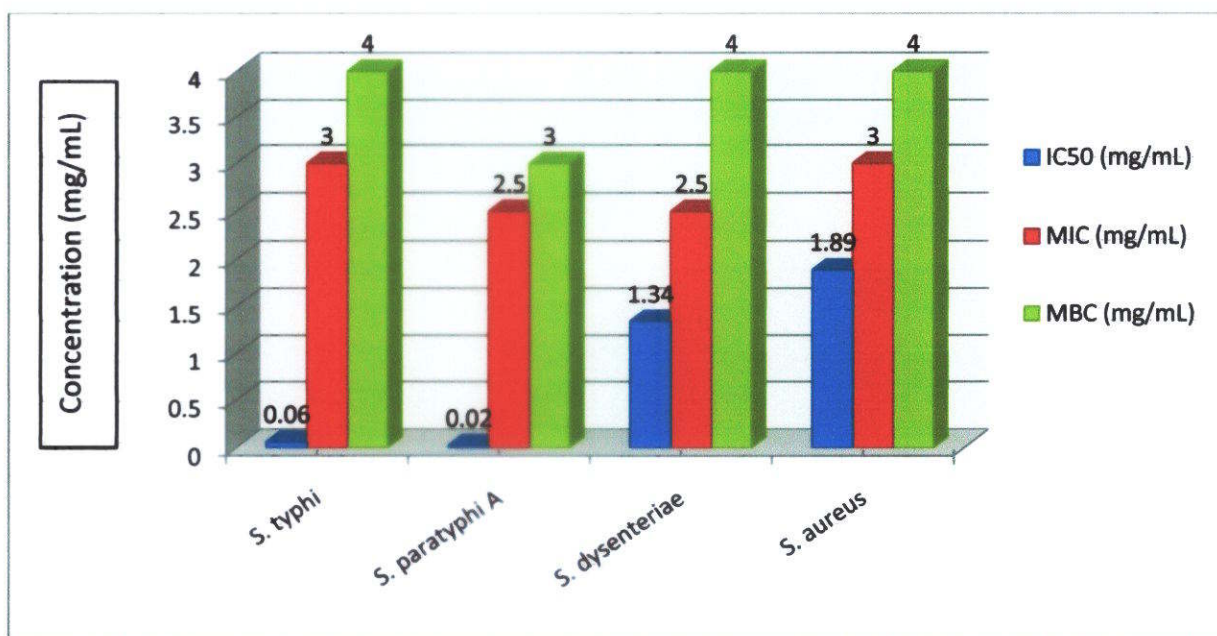


Figure 23: IC₅₀, MIC and MBC values for test bacteria by methanol fraction of *S. cumini*

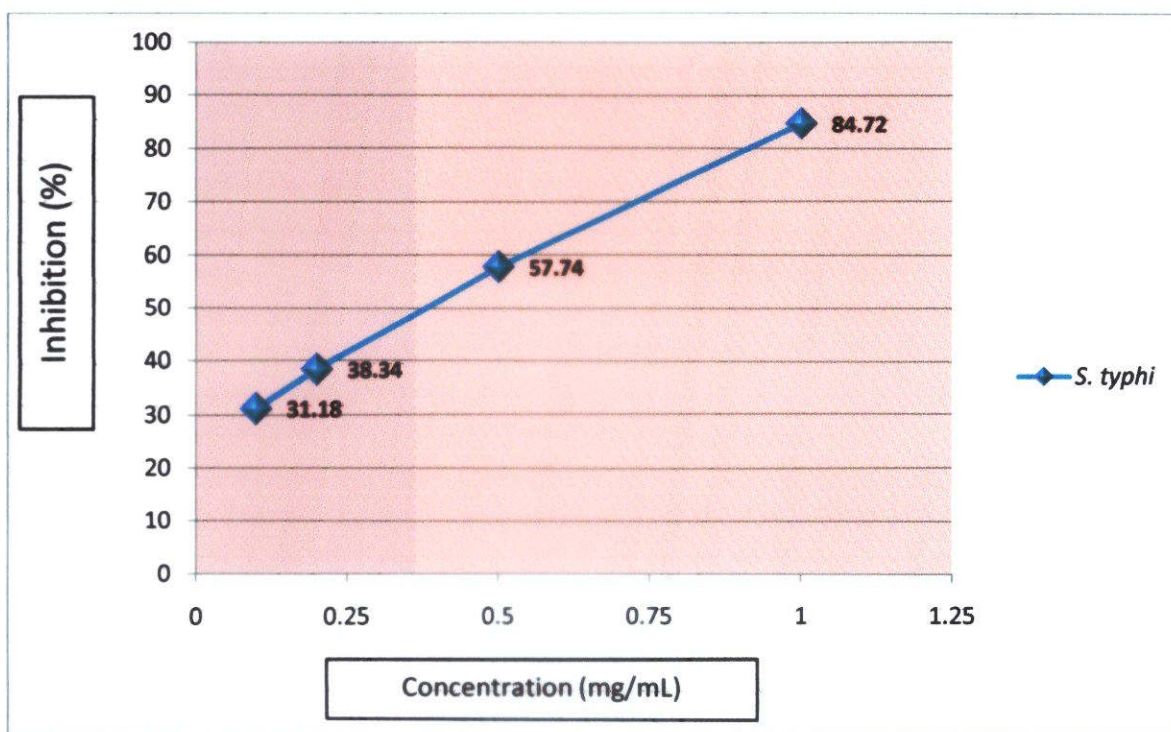


Figure 24: Dose dependent inhibition (%) of *S. typhi* by methanol fraction of *G. sylvestre*

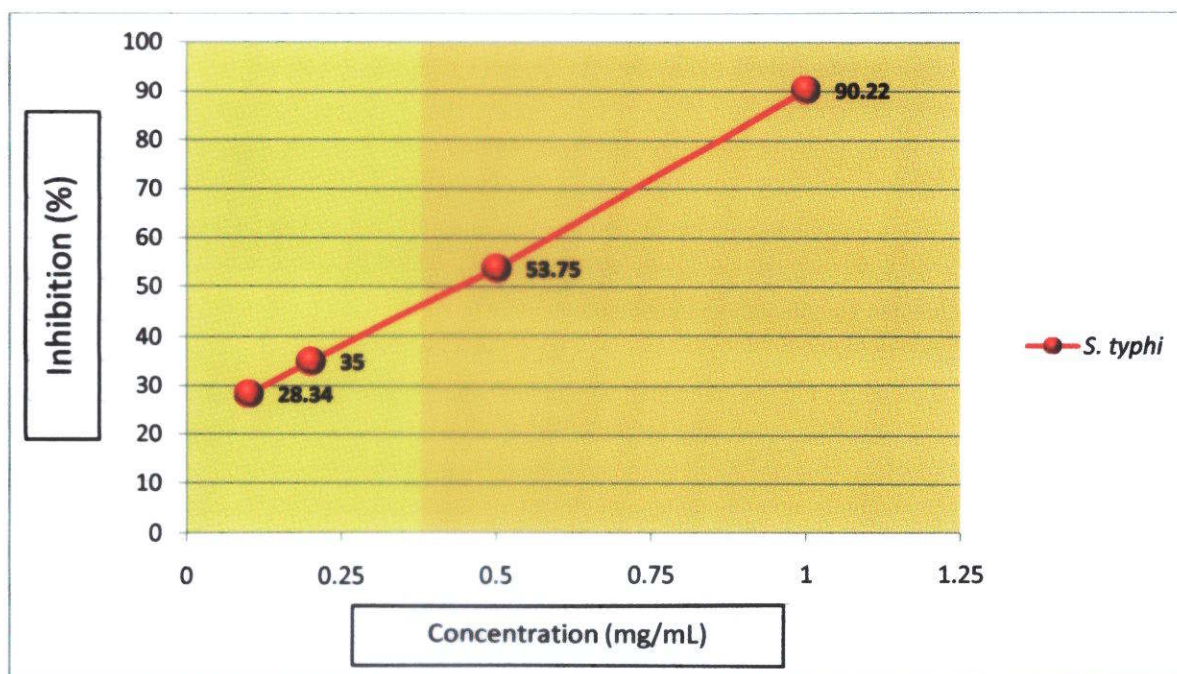


Figure 25: Dose dependent inhibition (%) of *S. typhi* by methanol fraction of *S. chirata*

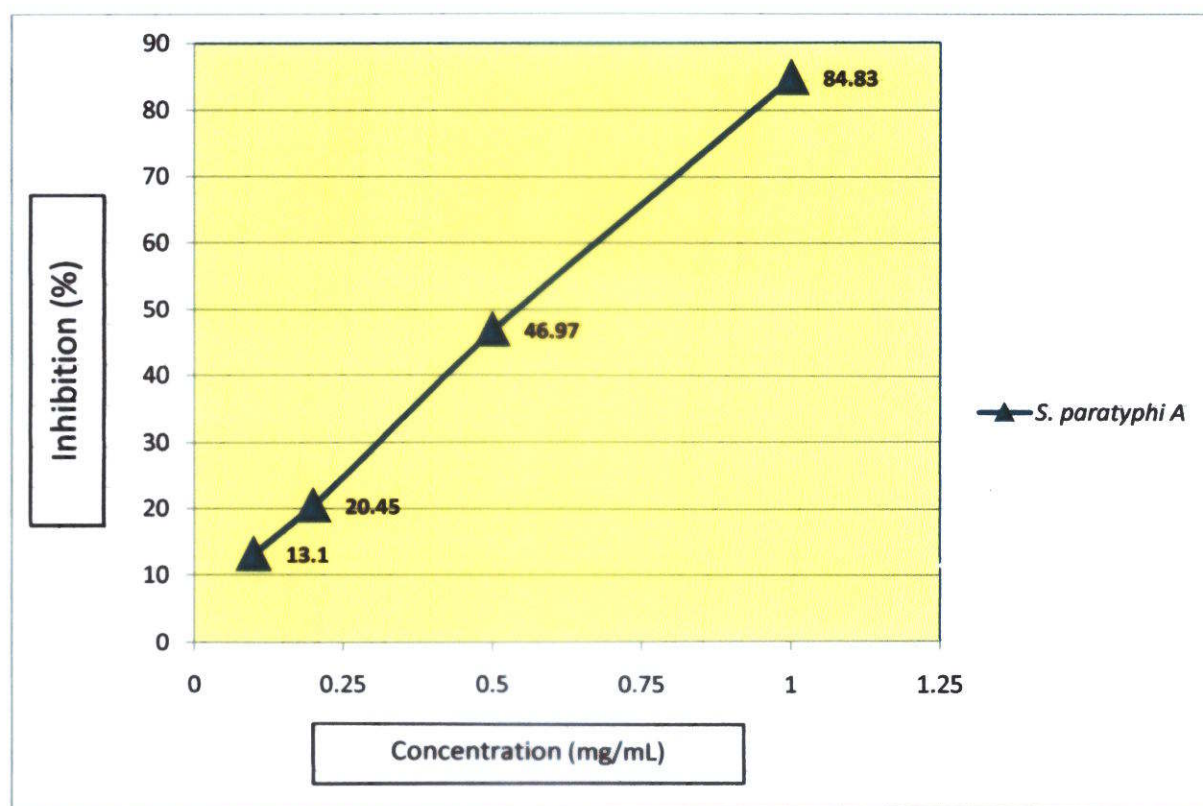


Figure 26: Dose dependent inhibition (%) of *S. paratyphi A* by methanol fraction of *C. indica*

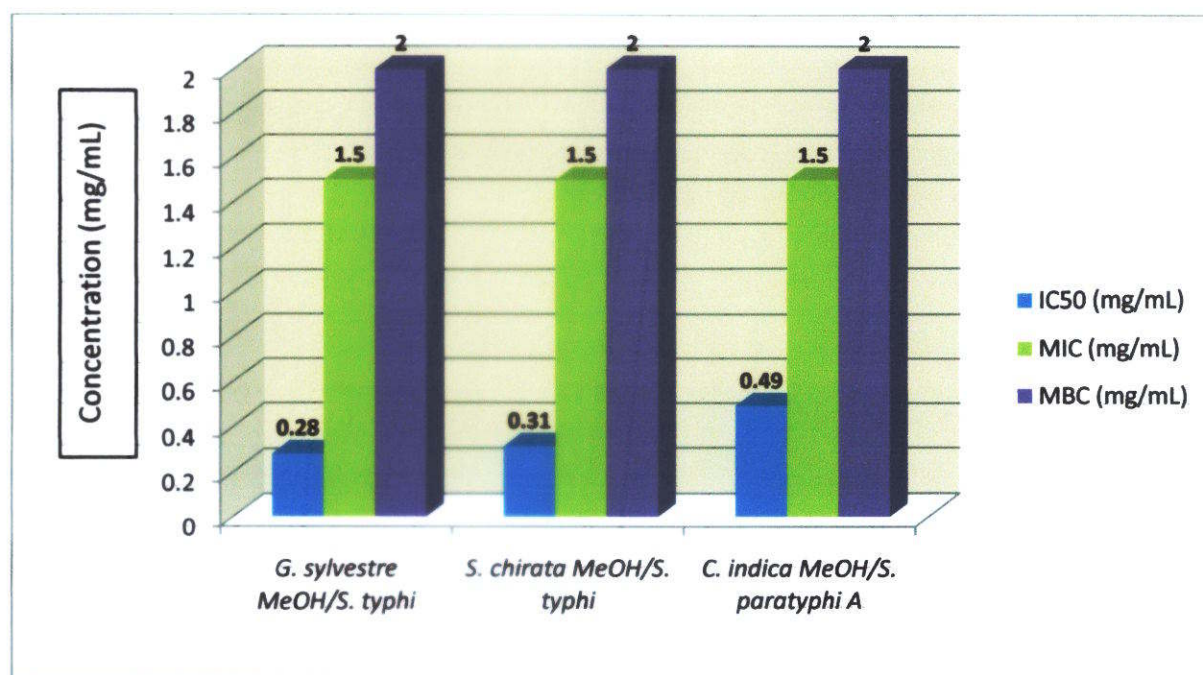


Figure 27: IC₅₀, MIC and MBC values for *S. typhi* by methanol fraction of *G. sylvestre*, *S. chirata* and for *S. paratyphi A* by methanol fraction of *C. indica*



Figure 28: Vials with doses of methanol fraction of *S. chirata* against *S. typhi*

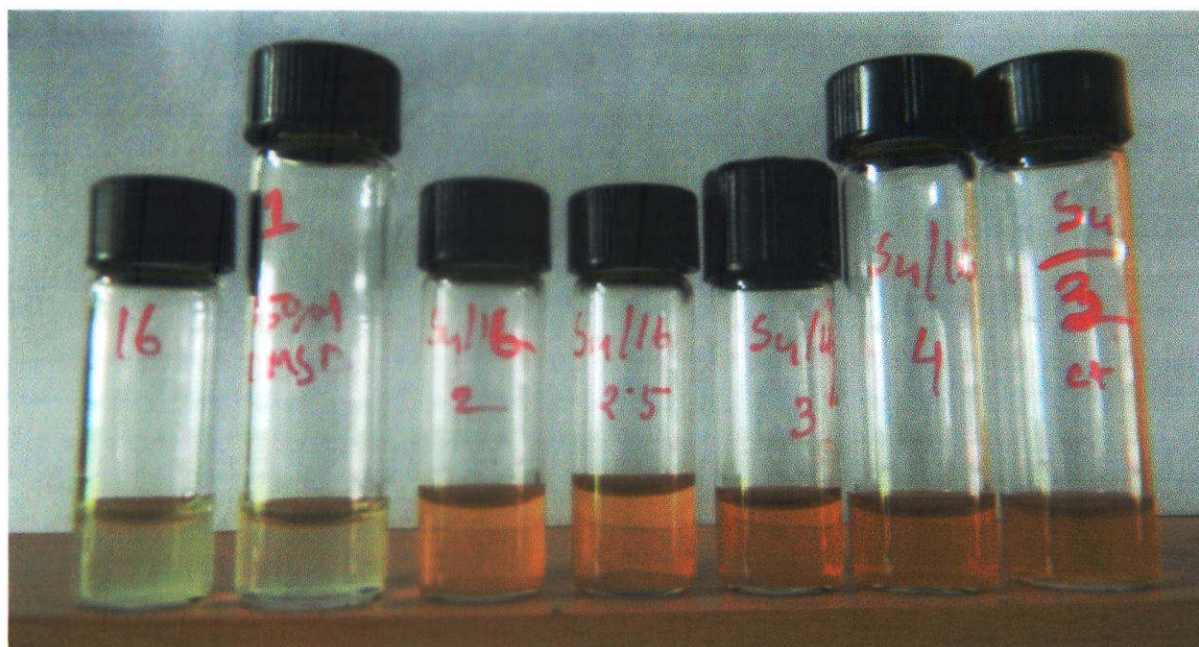


Figure 29: Vials with doses of methanol fraction of *S. cumini* against *S. aureus*



Figure 30: Vials with doses of methanol fraction of *G. sylvestre* against *S. typhi*

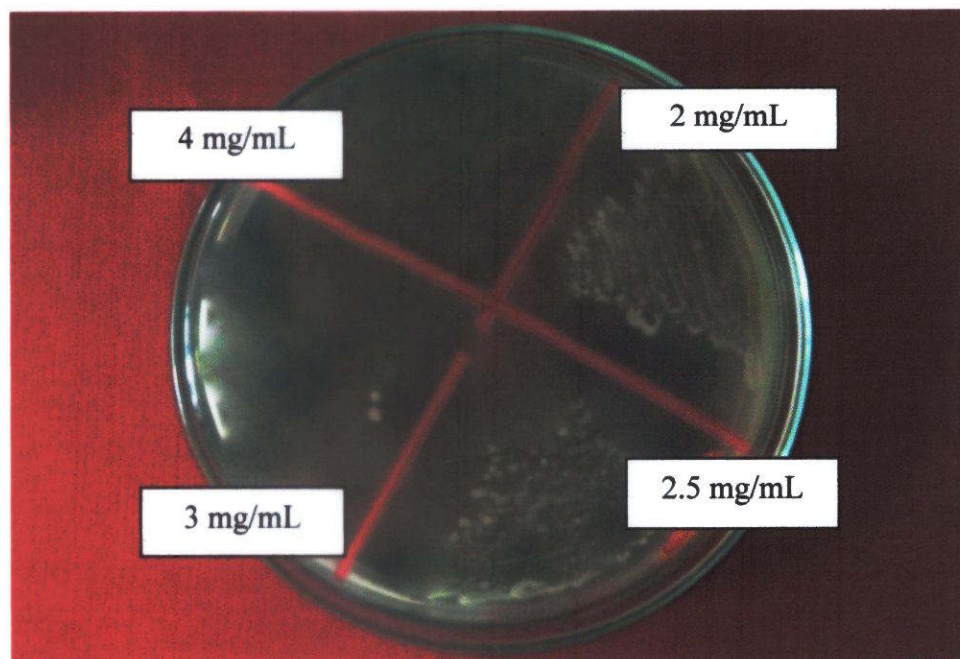


Figure 31: Plate for screening MBC for *S. aureus* by methanol fraction of *S. cumini*

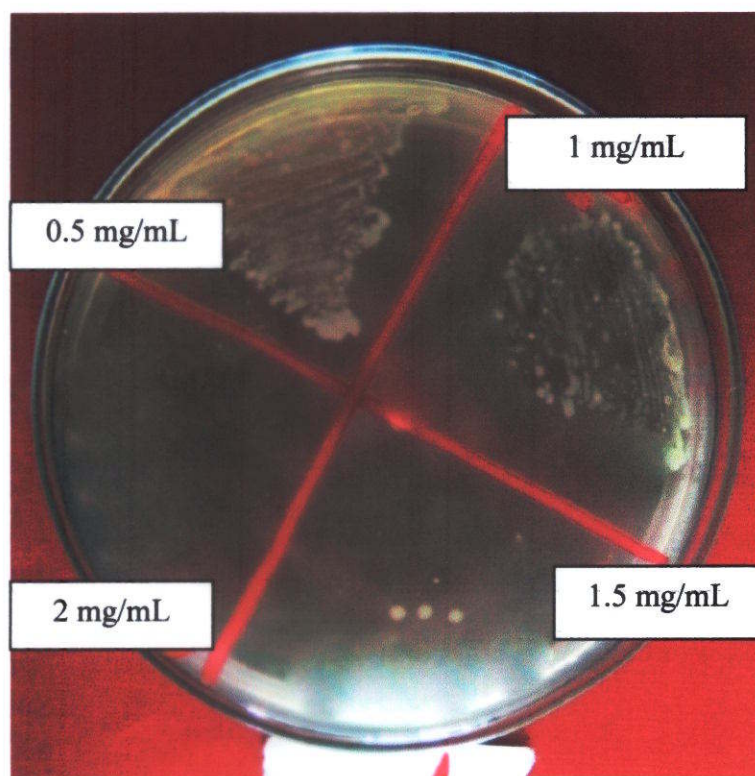


Figure 32: Plate for screening MBC for *S. typhi* by methanol fraction of *G. sylvestre*

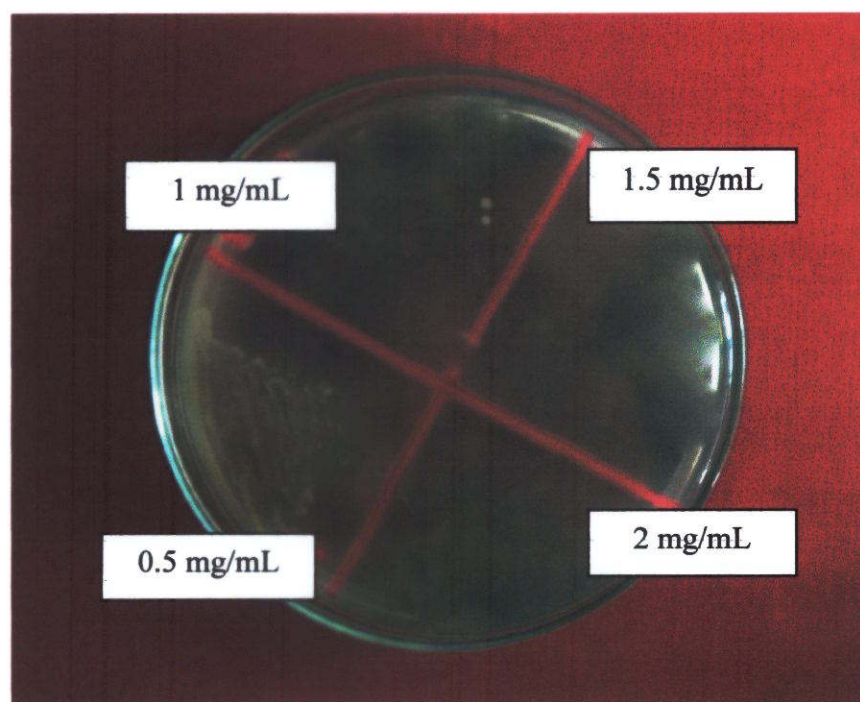


Figure 33: Plate for screening MBC for *S. typhi* by methanol fraction of *S. chirata*

Chapter Six

DISCUSSION

DISCUSSION

Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body (Edeoga et al., 2005). Different folk plant preparations are being prescribed by the traditional practitioner and also accepted by the users for diabetes like for any other diseases in many countries especially in third world countries. Now-a day more than 400 plants are being used in different forms for hypoglycemic effects (Wadkar et al., 2008). Medicinal plants produce bioactive compounds which act on different physiological systems and/or act through interfering in the metabolism of microbes infecting the hosts. The microbes may be pathogenic or symbiotic. In either way the bioactive compounds from medicinal plants play a determining role in regulating host-microbe interaction in favour of the host. So the identification of bioactive compound in plants, their isolation, purification and characterization of active ingredients in crude extracts by various analytical methods is important (Cowman, 1999; Adesokan et al., 2008).

Contrary to common belief, the association between diabetes mellitus and increased susceptibility to infection in general is not supported by strong evidence (Wheat, 1980). However, many specific infections are more common in diabetic patients, and some occur almost exclusively in them. Other infections occur with increased severity and are associated with an increased risk of complications in patients with diabetes. It remains uncertain whether diabetes is an independent risk factor for an increased incidence or severity of common upper or lower respiratory tract infections (Koziel and Koziel, 1995; Woodhead et al., 1987). Two patterns of susceptibility to pneumonia in patients with diabetes have been noted. Infections caused by certain microorganisms (*Staphylococcus aureus*, gram negative organisms, and *Mycobacterium tuberculosis*) occur with increased frequency. Infections due to other microorganisms (*Streptococcus pneumonia* and influenza virus) are associated with increased mortality and morbidity (Bouter et al., 1991; Marrie, 1992). Diabetes may also predispose patients to more severe infections of the upper urinary tract; the upper tract is involved in up to 80 percent of urinary tract infections in diabetic patients. Complications also occur more frequently in diabetic patients than in non-diabetic patients with established urinary tract

infections (Joshi et al., 1999). Foot infections are the most common soft-tissue infections in patients with diabetes. Potential complications include osteomyelitis, amputation, and death. Surgical debridement of all devitalized tissue is essential, and a multidisciplinary approach to the treatment of foot infection is recommended. Among nonpedal soft-tissue infections, necrotizing fasciitis is the most important. The associated mortality is approximately 40 percent (Gozal et al., 1986; Ahrenholz, 1988; Sentochnik, 1995). The most common locations are the arms and legs and the abdominal wall. Combination of anaerobes, facultative aerobes, strict anaerobes such as *Bacteroides fragilis*, *E. coli* or *Clostridium* species necrotizing fasciitis (Gozal et al., 1995). A study of bacteremia caused by *Staphylococcus aureus* found no difference in mortality between diabetic and nondiabetic patients (Cooper and Platt, 1982). In spite of the presence of known antidiabetic medicine remedies from medicinal plants are used with success to treat these diseases (Carson and Riley, 2003).

In this study, disc diffusion method was applied to test the extracts. At first MeOH extracts of thirteen plants were subjected for screening against six pathogenic bacteria. Among the extracts at 400 µg/disc, *C. indica*, *G. sylvestre*, *S. chirata* and *S. cumini* were lifted up with considerable zone of inhibition. *C. indica* showed up with a zone of inhibition of 17.5 ± 0.71 mm against *S. paratyphi* (Table 4) which exceeded the result of standard antibiotic (Tetracyclin) and is quite illustrative in comparison to the findings of Hussain et al. (2010) that is ethanol and aqueous extracts of *C. indica* can inhibit *Salmonella* species. On the otherhand, *G. sylvestre* and *S. chirata* both exhibited strong activity against *S. typhi*. The zone of inhibition was recorded 18 ± 0.71 mm for *G. sylvestre* which was found three times stronger than the result of Nataranjan et al. (2011) and in case of *S. chirata* the result was 14 ± 1.41 mm and which was nearly double than that of Alam et al. (2009). Among these four plants, *S. cumini* extract conferred the strongest action against four of the test bacteria (*S. typhi*, *S. paratyphi* A, *S. dysenteriae* and *S. cumini*). The recorded results were 13 ± 0.71 mm, 11.5 ± 0.71 mm, 14.75 ± 0.35 mm and 8.75 ± 0.35 mm for *S. typhi*, *S. paratyphi* A, *S. dysenteriae* and *S. aureus* respectively (Table 4). Duraipandiyan et al. (2006) reported a lesser activity against *S. aureus* with a much greater concentration. After this preliminary screening, extracts were subjected to evaluate IC₅₀ values. A range of concentrations were applied to estimate the percentage of inhibition by computing the ODs. *C. indica* confirmed 5.47 mg/mL as IC₅₀ against *S. paratyphi* A (Table 6). Hussain et al., (2010) found that

ethanolic extracts of *C. indica* can inhibit *Salmonella* and *Staphylococcus* species. However, *S. typhi* showed susceptibility against *G. sylvestre* (6.37 mg/mL). Yogisha and Raveesha, (2009) reported that, MeOH extract of *G. sylvestre* leaves can inhibit *S. typhi*. Moreover, *S. cumini* showed a notable achievement by inhibiting *S. dysenteriae* (8.65 mg/mL) and *S. aureus* (8.99 mg/mL). Narendhirakannan and Banerjee (2011) found a better action of MeOH extract of *S. cumini* against *S. aureus*.

Considering the previous data *C. indica*, *G. sylvestre*, *S. chirata* and *S. cumini* were fractionated using five different solvents (hexen, diethylether, chloroform, ethylacetate and methanol). Among the five fractions only methanol fraction was found as potent to some extent. As shown in previous data, *S. cumini* was again on the top among the tested fractions by inhibiting the four bacteria (Table 5). An identical number was demonstrated by Ratnam and Raju (2008) against *S. aureus* by MeOH fraction of *S. cumini*. It was reported that, different extracts of *S. cumini* leaves also possess strong antibacterial activity (Oliveira et al., 2007; Ahsan et al., 2011). However, among the assorted fractions, MeOH fraction of *S. cumini* showed low IC₅₀ values. Among the results, 20 µg/mL was calculated as IC₅₀ for *S. paratyphi* A, which was 60 µg/mL for *S. typhi*. Broad spectrum antibacterial potency of MeOH fraction of *S. cumini* seed was proved by Ratnam and Raju, (2008) in addition to its vibriocidal (Acharyya et al., 2009) and anticancer aptitude (Afify et al., 2011). Subsequently, *G. sylvestre* and *S. chirata* both presented similar kind of inhibition against *S. typhi*. Saneja et al. (2010) affirmed that methanolic extract of *G. sylvestre* leaves showed moderate activity against pathogenic *Salmonella* species and Laxmi et al. (2011) concluded with similar susceptibility of *S. typhi* against *S. chirata*. In alliance, *C. indica* inhibited *S. paratyphi* A at lower concentrations while Dewanjee et al. (2007) reported inhibitory effect of *C. indica* against *S. aureus* and *S. dysenteriae*. Over and above this, MeOH fractions of these four plants were again investigated to seek out the MIC and MBC values against respective bacteria. *S. cumini* showed up with the lowest MIC (2.5 mg/mL) and MBC (3 mg/mL) against *S. paratyphi* A. This result is much lower than that of Udayan et al. (2006). Surprisingly, *G. sylvestre*, *S. chirata* and *C. indica* showed matching MIC (1.5 mg/mL) and MBC (2 mg/mL) values against *S. typhi* and *S. paratyphi* A respectively. These results showed a little variation from the study of Kalidass, and Mohan, (2010), Sultana et al. (2007), and Umbreen et al. (2008).

Assessing all data it can be stemmed that among the antidiabetic plants used, *S. cumini* (seed) showed the best activity against the tested pathogenic bacteria. Thus, it would be an excellent herbal remedy for both diabetes as well as bacterial infections.

The results of this investigation revealed that methanol extracts of four of the plants possess differentiating antibacterial activity against selected bacterial strains which encourages developing novel broad spectrum antibacterial formulation in future. Thus, in search of potent antibacterial agent, formulation comprising different proportions of these extracts may be proven good. This study has not only shown the scientific basis of therapeutic uses of traditional plants, but also confirmed the ethnomedicinal claims for the selected plants.

Chapter Seven

CONCLUSION

CONCLUSION

Thomas Jefferson wrote that “The greatest service which can be rendered any country is to add a useful plant to it’s culture.” Plants have forever been a catalyst for our healing. In order to halt the emerging infectious diseases, it will require a multi-pronged approach that includes the development of new drugs. However, the interest in herbal drug research continues with an expectation that some day we would be able to bring a safer and effective compound with all the desired parameters of a drug that could replace the synthetic medicines. Therefore, this research was to evaluate antibacterial activity of common antidiabetic plants in Bangladesh. Methanol extracts of *C. indica*, *G. sylvestre*, *S. chirata* and *S. cumini* came up with excellent proof of inhibition against *S. typhi*, *S. paratyphi* A, *S. aureus* and *S. dysenteriae*. In addition, methanol fractions of the four plants were also found active against the same four bacteria. However, *S. cumini* (seed) was found distinctive with excellent antibacterial potency among all the tested plants. The results thus raise the notion that, a range of common antidiabetic plants in Bangladesh have proficient antibacterial action. People with diabetes mellitus are more susceptible to numerous bacterial infections. Hence, assessing the findings of this study it can be recommended that patients with diabetes and diabetes associated bacterial infections should prefer *S. cumini* (seed) as herbal remedy. This would be encouraging to the certain degree for the traditional medicinal uses of these plants and this effort can reinforce the concept of ethnobotanical approaches to drug discovery through screening for potential source of bioactive substances. Research on medicinal plants in each geographical region need to be conducted for proper integration of these remedies in the modern system of medicine for providing benefit of effective and cheaper drugs from the plant resource available in Bangladesh.

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APPENDIX

APPENDIX

$$1. \% \text{ of inhibition} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100\%$$

Table 2: Yield Percentage of fractionated extract

Solvent	Powder Used	Yield (%)			
		<i>C. indica</i>	<i>G. sylvestre</i>	<i>S. chirata</i>	<i>S. cumini</i>
Hexen	10 g	1.26	0.39	0.94	0.72
Diethylether		0.84	0.49	0.88	0.26
Chloroform		0.27	0.24	0.06	0.05
Ethylacetate		0.81	0.3	0.38	0.07
Methanol		1.34	3.74	2.33	2.29

Table 6: Percentage of Inhibition and IC₅₀ values

<i>C. indica, Salmonella paratyphi A</i>		
Conc.(mg/mL)	Inhibition (%)	IC ₅₀ (mg/mL)
2	23.04 ± 0.37	5.47
4	36.45 ± 0.84	
8	67.39 ± 0.96	
10	75.1 ± 0.88	
<i>G. sylvestre, Salmonella typhi</i>		
Conc.(mg/mL)	Inhibition (%)	IC ₅₀ (mg/mL)
0.5	10.12 ± 0.05	6.37
1	14.60 ± 0.08	
2	22.11 ± 0.24	
4	35.49 ± 0.5	
6	47.76 ± 0.97	
8	55.94 ± 1.48	
10	75.84 ± 1.72	
<i>S. cumini, Shigella dysenteriae</i>		
Conc.(mg/mL)	Inhibition (%)	IC ₅₀ (mg/mL)
2	10.05 ± 0.14	8.65
4	24.84 ± 0.26	
8	41.54 ± 0.06	
10	66.84 ± 0.22	
<i>S. cumini, Staphylococcus aureus</i>		
Conc.(mg/mL)	Inhibition (%)	IC ₅₀ (mg/mL)
2	2.17 ± 0.02	8.99
4	23.62 ± 0.09	
8	36.28 ± 0.24	
10	62.17 ± 0.27	



Table 7: IC₅₀, MIC an MBC result of fractionated sample

<i>S. cumini</i> MeOH, <i>S. typhi</i>				
Conc.(mg/mL)	Inhibition (%)	IC ₅₀ (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
0.01	36.26 ± 1.68	0.06	3	4
0.05	50 ± 0.29			
0.1	63.94 ± 0.304			
0.2	65.47 ± 0.64			
0.5	68 ± 2.87			
1	72.88 ± 0.74			
2	90.23 ± 2.04			
3	100			
4	100			
<i>S. cumini</i> MeOH, <i>S. paratyphi</i> A				
0.001	33.97 ± 0.87	0.02	2.5	3
0.005	45.62 ± 0.74			
0.01	55.76 ± 2.02			
0.05	62.49 ± 0.55			
0.1	67.41 ± 1.12			
0.2	68.5 ± 2.22			
0.5	72.28 ± 0.76			
1	77.60 ± 2.98			
2	92.97 ± 0.61			
3	100			
4	100			
<i>S. cumini</i> MeOH, <i>S. dysenteriae</i>				
0.1	7.55 ± 0.07	1.34	2.5	4
0.2	8.09 ± 0.1			
0.5	12.56 ± 0.14			
1	21.53 ± 0.19			
2	55.78 ± 0			
3	100			
4	100			
<i>S. cumini</i> MeOH, <i>S. aureus</i>				
0.1	0.7 ± 0.01	1.89	3	4
0.2	5.72 ± 0.01			
0.5	12.21 ± 0.07			
1	24.83 ± 0.37			
2	55.86 ± 1.78			
3	100			
4	100			