

COMPARISON OF PHENOLICS AND ANTIBACTERIAL ACTIVITY OF COMMONLY USED ANTIDIABETIC MEDICINAL PLANTS IN BANGLADESH

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Abstract

In the present study, phenolic contents and antibacterial activity were compared among the medicinal plants commonly used to treat diabetes in Bangladesh. Folin-Ciocalteu's method was used to determine total content of phenolics (TPH) in crude methanol extracts. Disc diffusion, tube dilution and spread plate methods were used to study bacterial susceptibility, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), respectively. *Syzygium cumini* had the highest content of polyphenols, 294.5 mg gallic acid equivalent (GAE)/g extract, followed by *Swertia chirata* (183.1 mg GAE/g extract) and *Ficus racemosa* (154.2 mg GAE/g extract). Among the crude methanol extracts, *S. cumini* showed strong inhibition against *Salmonella paratyphi* A, *S. typhi*, *Shigella dysenteriae* and *Staphylococcus aureus* in disc diffusion test at the concentration of 400 µg/disc. At this concentration, crude methanol extracts of *Coccinia indica*, *Ficus racemosa*, *Gymnema sylvestre* and *Swertia chirata* also inhibited *S. paratyphi* A, *Escherichia coli*, *S. typhi* and *S. typhi*, respectively whereas no extract inhibited *Vibrio cholerae*. Since *C. indica*, *G. sylvestre*, *S. chirata* and *S. cumini* exhibited strong inhibition, they were successively fractionated into *n*-hexane, diethyl ether, chloroform, ethyl acetate and methanol. Among them, methanol fractions profoundly inhibited the growth of respective bacteria. Thereafter, their IC₅₀, MIC and MBC values were determined. The results revealed that among the commonly used antidiabetic medicinal plants, *S. cumini* possessed the highest content of polyphenols as well as the strongest antibacterial activity. Therefore, *S. cumini* could be used to prepare herbal medicine or supplement to treat both diabetes and associated bacterial infections.

Key words: Antibacterial activity, antidiabetic plants, diabetes, medicinal plants, pathogenic bacteria, phenolics, *Staphylococcus aureus*

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder of carbohydrates, proteins, and fats characterized by hyperglycemia resulting from malfunction in insulin secretion and/or insulin action. Chronic hyperglycemia produces toxicity of glucose that in turn, accelerates production of reactive oxygen species (ROS). ROS is responsible for damaging cellular proteins, membrane lipids and nucleic acids. Hence lipid peroxidation is another feature of chronic diabetes. Therefore, diabetic patients experience various severe complications such as atherosclerosis, coronary heart disease, diabetic nephropathy, neuropathy and retinopathy [1, 2]. Besides organ complications, diabetic patients also suffer more frequently from (complicated) infections compared to nondiabetic patients [3] and are at increased risk for lower respiratory tract infection, urinary tract infection, and skin and mucous membrane infection [4]. Hence diabetic adults are at greater risk for infection-related mortality [5]. Reportedly, poor control of diabetes, impaired leukocyte function, and various host factors all contribute to the pathogenesis of microbial infection in the diabetic patient. Diabetes has been identified as a risk factor for infection with *Salmonella enteritidis* [6] as well as the incidence of tuberculosis among persons with diabetes was found to be three or four times as high as in the general population [7]. Aerobic gram-positive cocci are the predominant pathogens in diabetic foot infections, especially *Staphylococcus aureus*. But patients with chronic wounds experience polymicrobial infections caused by β -hemolytic streptococci, enterococci, *Bacteroides fragilis* and some enteric gram-negative rods and obligate anaerobes. However, majority of infections in diabetic patients are localized in the urinary tract [8]. *E. coli* is the most common causative uropathogen in 47% of the urinary tract infections (UTI) in diabetic patients and in 68% of the UTIs in non-diabetic patients [9, 10]. It is estimated that 40-50% of women and 5% of men will develop UTI in their lifetime [11]. Other uropathogens found in patients with diabetes mellitus, include *Klebsiella* spp, *Enterobacter* spp, *Proteus* spp, Group B Streptococci and *Enterococcus faecalis* [12, 13].

Medicinal plants constitute an important natural wealth and they provide primary health care services to more than 80% of the world population. 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 based therapies as prescribed by Ayurvedic and other indigenous systems of medicine have been well adapted. Our survey on herbal markets in Bangladesh showed that only 14-16 antidiabetic medicinal plants namely *Achyranthes aspera*, *Adhatoda zeylanica*, *Aegle marmelos*, *Alpinia nigra*, *Asparagus racemosus*, *Andrographis paniculata*, *Bacopa monniera*, *Coccinia indica*, *Ficus racemosa*, *Gymnema sylvestre*, *Momordica chinensis*, *Swertia chirata*, *Swietenia mahagoni*, *Syzygium cumini* and *Trigonella foenum-graecum* are available and people randomly use these plants to treat diabetes. Various reports [14, 15, 16] described their hypoglycemic or antidiabetic effects. They are also used to treat asthma, bronchitis, cough, dyspepsia, epilepsy, hemorrhoids, inflammation, jaundice, leprosy, rheumatism and/or ulcer etc. diseases [15, 16]. These antidiabetic medicinal plants are rich in alkaloids, essential oils, flavonoids, glycosides, phenolic compounds, saponins, sterols, tannins etc. [15]. In spite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from these medicinal plants are used with success to treat diabetes. Since chronic hyperglycemia and lipid peroxidation are two major characteristic features of diabetes, it is essential to develop hypoglycemic agents coupled with antioxidant activity. In previous paper, we explored antioxidant potentiality of these traditionally used antidiabetic medicinal plants [17]. However, along with various complications diabetes acts as a risk factor for some infectious diseases. Thus for preventing or reserving diabetic complications, it is also needed to identify potential antidiabetic medicinal plants that have substantial amount of antihyperglycemic, antioxidant and antimicrobial components. These components either act on different systems in man, and/or act through interfering in the metabolism of microbes infecting them. Hence the bioactive components from medicinal plants play a determining role in regulating host-microbe interaction in favor of the host [18].

However, polyphenols show various biological activities like antioxidative, antimicrobial, antidiabetic, antiallergic etc. Reportedly, some specific infections are more common in diabetic patients; even occur almost exclusively such as infections with the species of *Escherichia*, *Mycobacterium*, *Salmonella*, *Staphylococcus* and *Streptococcus* are predominant in diabetic patients. In addition, diabetic patients also suffer with abdominal complications and diarrhea. Till now, no paper compares antibacterial activity of these antidiabetic medicinal plants. Therefore, in the present study, we have investigated polyphenol content and antibacterial activity of 13 antidiabetic medicinal plants that are commonly used to treat diabetes in Bangladesh

Material and Methods

Plant samples

Depending on our market survey results and various literature, 13 commonly used antidiabetic medicinal plant samples (fruits, leaves, roots, seeds and/or stems) namely *Achyranthes aspera* (leaf), *Adhatoda zeylanica* (leaf), *Alpinia nigra* (rhizome), *Asparagus racemosus* (root), *Bacopa monniera* (leaf), *Coccinia indica* (leaf), *Ficus racemosa* (bark), *Gymnema sylvestre* (leaf), *Momordica chinensis* (fruit), *Swertia chirata* (whole plant), *Swietenia mahagoni* (seed), *Syzygium cumini* (seed) and *Trigonella foenum-graecum* (seed), were collected and cut into small pieces and shed-dried. The dried parts were ground into powder with the help of a grinder. The powders were stored separately in air tight containers and kept in a cool, dark, and dry place.

Preparation of crude methanol extract

The powder of each type of medicinal plant was placed separately in a clean, flat-bottomed glass container and soaked with methanol. Each container was sealed and kept for a period of 07 days with regular shaking and stirring of the contents followed by their filtration and evaporation. The % yield of the final methanol extracts for *A. aspera* (leaf), *A. zeylanica* (leaf), *A. nigra* (rhizome), *A. racemosus* (root), *B. monniera* (leaf), *C. indica* (leaf), *F. racemosa* (bark), *G. sylvestre* (leaf), *M. chinensis* (fruit), *S. chirata* (whole plant), *S. mahagoni* (seed), *S. cumini* (seed) and *T. foenum-graecum* (seed) were found to be

3.45, 7.52, 4.36, 20.00, 6.00, 3.24, 8.52, 4.17, 5.84, 7.65, 15.56, 9.36 and 8.08 % of dry weight of the powder respectively. Twenty milligram (20 mg) of each extract was dissolved in 1 mL of methanol (20 mg/mL) to prepare stock-concentration for the experiments.

Preparation of different fractions

Ten gram (10 g) of dried powder of each sample was soaked in 200 mL of *n*-hexane for 30 min with intermittent shaking. Then the hexane fraction was collected by filtering through Whatman No. 1 filter paper. The collected liquid was dried until a constant dry weight was obtained. After this, 200 mL diethyl ether was added to with the residues present on the filter paper and followed the same procedure to obtain diethyl ether fraction. The same procedure was followed to prepare chloroform, ethyl acetate and methanol fractions, respectively. The dried fractions were measured and stored at 4 °C. Twenty milligrams (20 mg) of prepared fraction was dissolved in 1 mL DMSO (20 mg/mL) to prepare stock-concentration for the experiments.

Determination of total phenolics (TPH)

The total concentration of phenolics (TPH) in crude methanol extracts was determined according to the Folin-Ciocalteu method [19] with gallic acid (GA) as the standard and expressed (mg) as gallic acid equivalents (GAE)/g of extract.

Bacterial species

To evaluate antibacterial activity, 6 pathogenic bacteria were collected from ICDDR'B (International Center for Diarrheal Disease Research' Bangladesh) namely, Gram-positive: *Staphylococcus aureus* and Gram-negative: *Escherichia coli*, *Salmonella paratyphi* A, *S. typhi*, *Shigella dysenteriae* and *Vibrio cholerae*.

Preparation of inocula

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 at 37 °C.

Preparation of the discs

Sterilized blank paper discs (6 mm in diameter, Oxoid Ltd.) were impregnated separately with the

stock-concentration (20 mg/mL) to get a final concentration of 400 µg/disc. The discs were placed in sterilized petriplates 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 showed any antibacterial activity. Tetracycline (Oxoid Ltd.) was used as standard antibiotic discs (30 µg/disc) for positive control.

Susceptibility tests

Disc diffusion method was used to know susceptibility of bacteria against the crude methanol extracts or different fractions of antidiabetic medicinal plants. 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 nutrient agar plates were prepared by pouring 20 mL of molten medium into sterile petriplates and were allowed to solidify. Then plates were seeded properly by pouring the culture broth with a Pasteur's pipette to make bacterial lawn. The excess culture broth was withdrawn from the plates and allowed to dry. Discs impregnated with extract or fraction (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.

Determination of minimum inhibitory concentration (MIC) and IC_{50}

The Minimum Inhibitory Concentration (MIC) of crude methanol extracts or different fractions was determined by the tube dilution method. In this method, an increasing in concentration of extract or fraction was added to different test tubes. Calculated amount of concentration was added from the stock (20 mg/mL) to the sterilized screw-capped one drum vials each containing 2 mL of nutrient broth to obtain seven different concentrations (0.2, 0.5, 1, 2, 4, 6, 8, 10 µg/mL respectively). Then 10 µl of freshly grown inocula (5×10^6 CFU/mL) was added to each vial. Additional vials were also included as Positive Control (PC): medium with inoculums; Growth (G):

medium with extract or fraction and inoculums; Negative Control (NC): medium with extract or fraction. All the tubes were incubated at 37 °C. After incubation, optical densities (ODs) were taken by spectrophotometer (Hitachi, U-2910) at 700 nm. At lowest concentrations, vials that showed no turbidity or ODs due to bacterial growth were selected as MIC of the extract or fraction. Using the data, % of inhibition and IC_{50} were calculated. All assays were carried out in triplicate.

$$\% \text{ of Inhibition} = [\text{PC} - (\text{G} - \text{NC})] / \text{PC} \times 100$$

Determination of minimum bactericidal concentration (MBC) of fractions

The minimum bactericidal concentration (MBC) of different fractions was determined by measuring the viability loss of bacterial culture with an increasing concentration of fractions. Vials containing different concentration of fractions were plated out on nutrient agar plate to test the viability of bacteria. No colony formation indicated the MBC concentration(s).

Results

Polyphenols content

Polyphenols are secondary metabolites of plants that show various beneficial health promoting activities in humans. Figure 1 shows polyphenols content in 13 crude methanol extracts of medicinal plants commonly used to treat diabetes in Bangladesh. Seeds of *S. cumini* had the highest amount of polyphenols (294 mg GAE/g extract) followed by *S. chirata* (183 mg GAE/g extract), *F. racemosa* (154 mg GAE/g extract) and *B. monniera* (63 mg GAE/g extract).

Susceptibility test

Disc diffusion method was used to find out the antibacterial activity of crude methanol extracts of antidiabetic plants commonly used to treat diabetes. The diameters of zone of inhibition were measured in mm and the results showed as average \pm SD of three replicates (Table 1). Among the crude methanol extracts, *S. cumini* potentially inhibited the growth of *S. paratyphi* A, *S. typhi*, *S. dysenteriae* and *S. aureus* with a zone diameter of 11.5 ± 0.7 , 13 ± 0.7 , 14.8 ± 0.4 , and 8.8 ± 0.4 mm, respectively at 400 µg/disc.

However, the most common uropathogen in diabetic patients is *E. coli*, which was inhibited by *Ficus racemosa* with a zone diameter of 9 mm. *C. indica* showed highest inhibition zone (17.5 ± 0.7 mm) against *S. paratyphi* A whereas *G. sylvestre* showed against *S. typhi* (18 ± 0.7) followed by *S. chirata* (14 ± 1.4).

However, powders of these four plants were successively fractionated into *n*-hexane, diethyl ether, chloroform, ethyl acetate and methanol fractions. These fractions (400 µg/disc) were used in susceptibility tests and only methanol fractions prominently inhibited the growth of test bacteria (data not shown).

Minimum inhibitory concentration (MIC) and IC₅₀

Depending on the results of susceptibility tests, dose-dependent inhibition of the growth of *S. dysenteriae* and *S. aureus* was studied with the crude methanol extract of *S. cumini* whereas that for *S. paratyphi* A and *S. typhi* by *C. indica* and *G. sylvestre*, respectively (Figure 2). The extracts dose-dependently inhibited the growth of the test bacteria and MIC values were determined. From the dose-dependent curve their inhibition concentrations 50 (IC₅₀) were also calculated. *C. indica* showed lowest IC₅₀ value of 5.5 mg/mL against *S. paratyphi* A whereas *G. sylvestre* showed 5.9 mg/mL against *S. typhi* while *C. cumini* had 8.6 and 11.3 mg/mL for *S. dysenteriae* and *S. aureus* respectively.

However, methanol fraction of *S. cumini* strongly dose-dependently inhibited the growth of *S. paratyphi* A, *S. typhi*, *S. dysenteriae* and *S. aureus* (Figure 3). Figure 4 shows dose-dependent inhibition of *S. paratyphi* A by the methanol fraction of *C. indica*. Methanol fractions of both *G. sylvestre* and *S. chirata* dose-dependently inhibited the growth of *S. typhi* (Figure 4), from the data MIC and IC₅₀ values were determined. *S. cumini* showed lowest IC₅₀ of 0.02 mg/mL against *S. paratyphi* A followed by 0.06 mg/mL against *S. typhi* (Table 2).

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 nutrient agar plates to check the viability and at a certain concentration no visible growth of bacteria was found onto the plate.

Thus MBC values were recorded (Table 2). *S. typhi*, *S. dysenteriae* and *S. aureus* were completely inhibited by methanol fraction of *S. cumini* at a concentration of 4 mg/mL whereas *S. paratyphi* A at 3 mg/mL. MBC value of *G. sylvestre* and *S. chirata* was 2 mg/mL for *S. typhi* whereas *C. indica* showed MBC value of 2 mg/mL for *S. paratyphi* A.

Discussion

Medicinal plants are valuable treasure of broad range of inherent active ingredients used predominantly in traditional systems of medicine. More than 80% of the world population still use traditional plant based medicines for their primary healthcare needs. In Bangladesh, about 250 species of plants are used for the preparation of traditional medicines and therapeutic purposes [15]. Ethno-botanical studies [15, 16] reported approximately 60 medicinal plants used traditionally to treat diabetes in Bangladesh. In our survey on herbal market in Bangladesh, we found only 14-16 antidiabetic medicinal plants are available and people randomly using them to treat diabetes. However, it is assumed that medicinal plants that possess potential antihyperglycemic agents coupled with substantial amount of antioxidative and antimicrobial, especially antibacterial agents could effectively prevent, ameliorate or reserve diabetic complications. It was recorded that among these 13 antidiabetic medicinal plants, *S. cumini*, *S. chirata* and *F. racemosa* had high content of polyphenols. In previous studies, we reported polyphenols content of various edible fruits [20] and plants [21], & mangroves [22, 23]. Polyphenols content of the seeds of *S. cumini* (294 mg GAE/g extract) was near to that of *Phyllanthus emblica* (Emblic Myrobalan, 339 mg GAE/g extract) [20] and seeds of *Sonneratia apetala* (300.1 mg GAE/g extract) [24]. However, the extraction of phenolic compounds is commonly achieved with methanol or aqueous methanol [25]. Reportedly, polyphenols act as antioxidants, antimicrobial, anti-hyperglycemic, inhibit key digestive enzymes, activate and/or increase insulin secretion and thus antidiabetic. Basar et al [17] showed very high antioxidative activity and reducing power of *S. cumini*, *S. chirata* and *F. racemosa*. Among the 13 antidiabetic medicinal plants, *S. cumini* showed the strongest antibacterial activity. However, methanol fraction

of *S. cumini* that was obtained by successive solvent extraction method showed strong dose-dependent inhibition of the growth, especially *S. paratyphi* A and *S. typhi* (Figure 3). It was because successive solvent extraction extracted the components delicately and resulted methanol fraction was probably composed with high content of antibacterial agents. It was also reported that methanol extract of *S. cumini* consists of high phenolics, flavonoids, anthocyanins and antioxidants [17], which may also have antibacterial activity. Antibacterial components in these antidiabetic plants may kill the bacteria or inhibit their growth. Table 2 showed minimum bactericidal concentrations of *C. indica*, *G. sylvestre*, *S. chirata* and *S. cumini*. Hossain et al [24] showed that methanol extract of seeds of *Sonneratia apetala* was functionally rich with antidiabetic, antioxidant and antibacterial compounds. The increasing prevalence of multidrug resistant strains of bacteria and the appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies [26]. It is notable that gram-negative bacteria are generally more resistant compared to the gram-positive ones [27] due to the significant differences in their outer layers [28] and the enzymes system in periplasmic space [29]. However, methanol fraction of *S. cumini* possessed antibacterial agents that effectively inhibited gram-positive, *S. aureus* and gram-negative, *S. paratyphi* A, *S. typhi*, and *S. dysenteriae*.

It was also reported that leaves and seeds of *S. cumini* also possess strong antibacterial activity [30, 31]. Ethanol and aqueous extracts of *C. indica* inhibited *Salmonella* species [32]. *G. sylvestre* and *S. chirata* inhibited the growth of *S. typhi* similar to the reported results [33, 34]. Ahmed et al [35] showed that bark extract of *F. racemosa* inhibited *S. aureus* and *E. coli*. Reportedly, alkaloids, flavonoids, phenols, saponins, steroids, tannins etc are secondary metabolites of plants and serve as defense molecules in adverse conditions, such as microbial infection. These phytochemicals either interfere with the synthesis of proteins, cell wall or cause leakage of proteins, enzymes and lipids of bacteria.

Among the antidiabetic medicinal plants used in this study, *S. cumini* (seed) showed the highest content of polyphenols as well as strong potentiality to inhibit the growth of tested pathogenic bacteria. Thus, it could be an excellent herbal remedy against bacterial infections.

Since *S. cumini*, *S. chirata* and *F. racemosa* have potential antidiabetic activity [14, 36, 37] and are composed of high antioxidants [17], they could be used for developing novel drug or supplements to treat diabetes and associated bacterial infections in the future.

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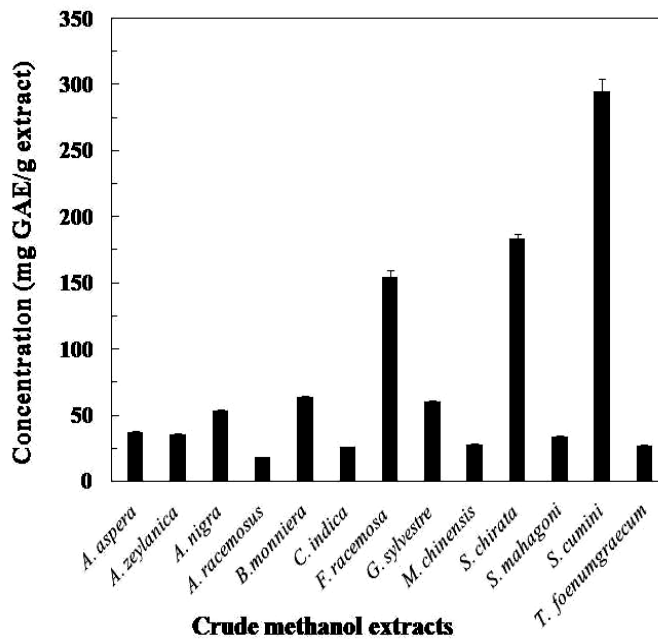


Figure 1. Polyphenols content in commonly used antidiabetic medicinal plants in Bangladesh. Values were expressed as mean \pm SD (bar); GAE means gallic acid equivalent

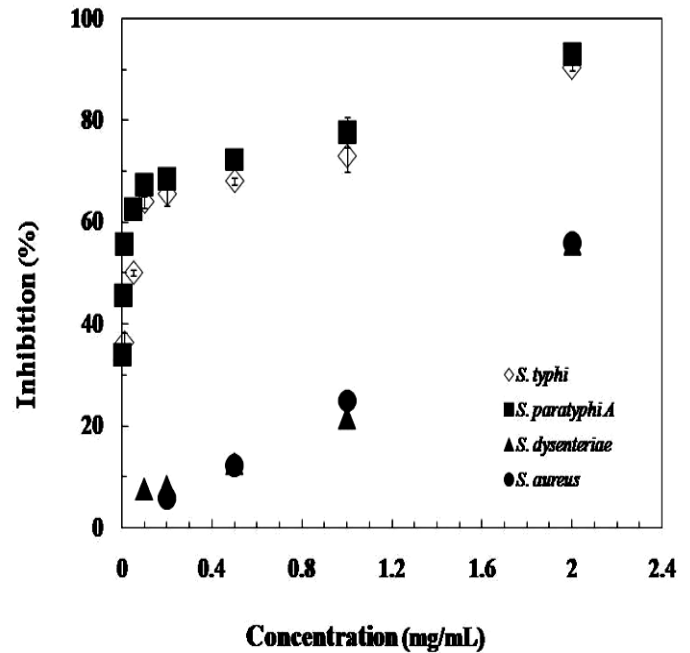


Figure 3. Dose-dependent inhibition (%) of four test bacteria by methanol fraction of *S. cumini*. Values were expressed as mean \pm SD (bar), n = 3

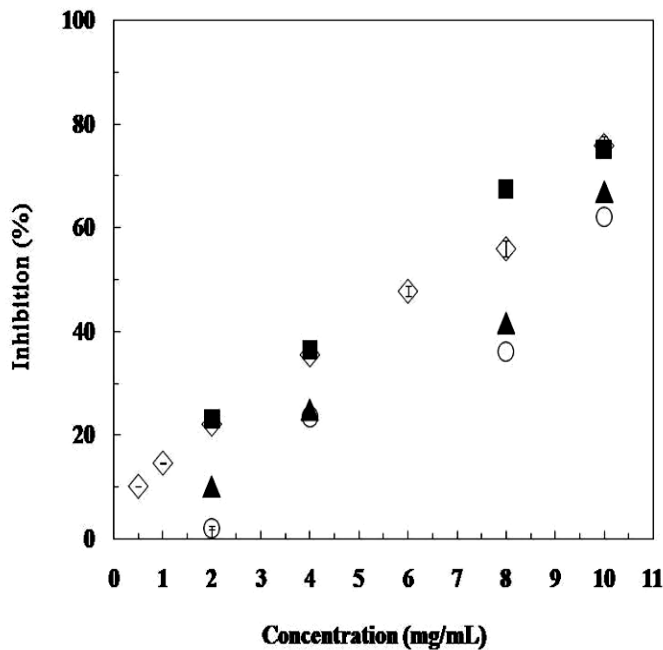


Figure 2. Dose-dependent inhibition (%) of *S. paratyphi A* (■), *S. typhi* (◇), *S. dysenteriae* (▲) and *S. aureus* (○) by crude methanol extract of *C. indica* (■), *G. sylvestre* (◇) and *S. cumini* (▲, ○). Values were expressed as mean \pm SD (bar), n = 3

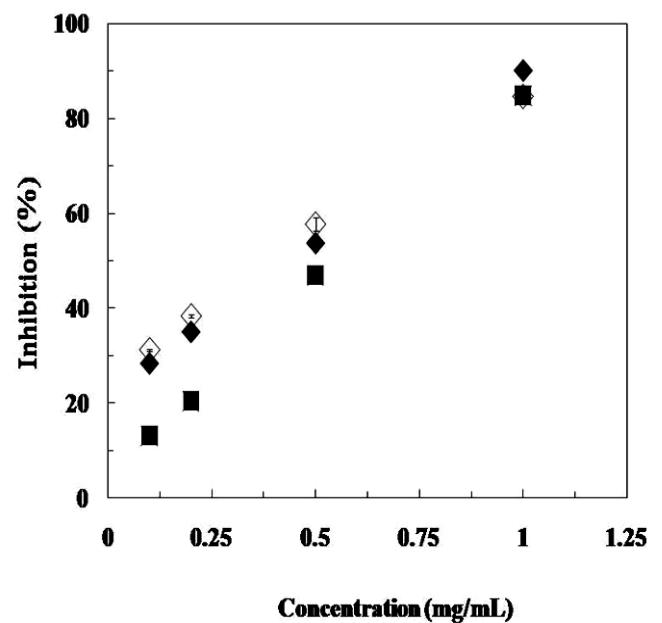


Figure 4. Dose-dependent inhibition (%) of *S. paratyphi A* (■) and *S. typhi* (◇, ◆) by methanol fraction of *C. indica* (■), *G. sylvestre* (◇) and *S. chirata* (◆). Values were expressed as mean \pm SD (bar), n = 3

Table 1. Mean diameter of zone of inhibition (mm ± SD) of methanol extracts at 400 µg/disc

Name of Bacteria	Aa	Az	An	Ar	Bm	Ci	Fr	Gs	Mc	Swc	Sm	Sc	Tfg	Tet
<i>E. coli</i>	7	6.5	7.5	-	6.5	7	9	6.8±4	7.3±4	7	-	-	-	16
<i>S. paratyphi A</i>	-	-	-	8.5	-	17.5±7	-	-	-	10	8.5	11.5±7	-	13
<i>S. typhi</i>	-	-	-	-	-	-	-	18±7	-	14±1.4	-	13±7	-	24.3±4
<i>S. dysenteriae</i>	-	-	6.5	7.3±4	-	-	8.5	-	-	-	8±1.4	14.8±4	8.3± 1.1	8
<i>S. aureus</i>	-	-	7.8±4	-	6.5	6.8±4	-	-	7±7	-	-	8.8±4	-	8.3±4
<i>V. cholera</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	19.8±4

Disc diameter: 6 mm; **Aa**: *A. aspera*, **Az**: *A. zeylanica*, **An**: *A. nigra*, **Ar**: *A. racemosus*, **Bm**: *B. monniera*, **Ci**: *C. indica*, **Fr**: *F. racemosa*, **Gs**: *G. sylvestre*, **Mc**: *M. chinensis*, **Swc**: *S. chirata*, **Sm**: *S. mahagonee*, **Sc**: *S. cumini*, **Tfg**: *T. foenum-graecum* and **Tet**: Tetracycline; n = 3, “-“ means no inhibition

Table 2. IC₅₀, MIC and MBC values of methanol fractions of four antidiabetic plants against test bacteria

Name of Bacteria	Name of methanol fraction (mg/mL)											
	<i>C. indica</i>			<i>G. sylvestre</i>			<i>S. chirata</i>			<i>S. cumini</i>		
	IC ₅₀	MIC	MBC	IC ₅₀	MIC	MBC	IC ₅₀	MIC	MBC	IC ₅₀	MIC	MBC
<i>S. paratyphi A</i>	0.49	1.5	2	-	-	-	-	-	-	0.02	2.5	3
<i>S. typhi</i>	-	-	-	0.28	1.5	2	0.31	1.5	2	0.06	3	4
<i>S. dysenteriae</i>	-	-	-	-	-	-	-	-	-	1.34	2.5	4
<i>S. aureus</i>	-	-	-	-	-	-	-	-	-	1.89	3	4

“-“ means not done

Author Proof