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IN VITRO COMPREHENSIVE ANALYSIS OF PHYTOCHEMICAL SCREENING, ANTIOXIDANT, ANTI-INFLAMMATORY AND ANTI-DIABETIC ACTIVITIES OF THREE DIFFERENT PLANTS OF BANGLADESH

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ABSTRACT

Medicinal plants are rich in phytochemicals have numerous biological activities including antioxidant, antidiabetic, anti-inflammatory, anticancer and antimicrobial activities. The present study was designed to investigate and compare the antioxidant, antidiabetic, anti-inflammatory, toxic properties and phenolic contents of methanol extracts from *Syzygium cumini* Bark (SCB), *Bombax ceiba* Fruits (BCF) and *Samanea saman* Flowers (SSF). Phytochemical screenings were also carried out. Among the extracts, SCB showed the highest antioxidant activity followed by BCF and SSF. Based on DPPH and Nitric oxide scavenging activity, the SCB extract was the most effective one with IC50 79.25 and 256. 3 µg/mL. The SCB extract had the most potent inhibitory activity against lipid peroxidation with IC50 317.2 µg/mL. In addition, the reducing capacity on ferrous ion was in the following order: SCB > BCF > SSF. The content of phenolics, flavonoids, flavonols and proanthocyanidins of SCB was found to be higher than other extracts. TPC and TFC were found strongly correlated ($P<0.01$) with antioxidant activities of the all plants extracts. In α-amylase inhibition assay SCB extract causes 53.95% inhibition at concentration 1000 µg/mL, which was significantly ($P<0.05$) different from another extracts. *In vitro* assay for anti-inflammatory activity test of BCF showed highest potency ($P<0.05$) in comparison to all other extracts. Alkaloids, flavonoids, phenols, carbohydrate, resins, saponins, steroids, tannins, anthracenosides and coumerins as phytochemicals were present in the extracts. The present study suggested that all the plants parts effectively ameliorate oxidative stress and thus be useful as pharmaceutical agents in various ailments. The present study also concluded that SCB have α-amylase inhibition and BCF have anti-inflammatory potential to be used as antidiabetic and anti-inflammatory agents.

KEYWORDS: Medicinal plants are rich in phytochemicals have numerous biological.

INTRODUCTION

Medicinal plants are the richest bio-resource of drugs and traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.[1] The active principles responsible for the therapeutic effects of medicinal plants are phytochemicals, usually secondary metabolites, including alkaloids, steroids, flavonoids, terpenoids and tannins etc.[2] The phytochemical compounds have a wide range of biological activities such as antioxidant activity, protection against coronary heart diseases, anti-inflammatory, anticancer and antimicrobial activities, which are caused by exposure to oxidative stress.[3,4] Natural antioxidants from plants offer an alternative source of dietary ingredients, For example, α-amylase, α-glucosidase inhibitors are considered as one of the effective measures for regulating type II diabetes by controlling glucose uptake and increasing secretion of insulin hormone.[5] On the other hand some phytochemicals including terpenoids, flavonoids, tanins have analgesic and anti-inflammatory activities.[6]

Syzygium cumini (L.) is a medicinal plant, whose parts were pharmacologically proved to possess hypoglycaemic, antibacterial, anti–HIV activity and anti-diarrhea effects.[7,8]

Bombax ceiba (L.) is commonly known as silk cotton tree.[9] Flower and bark of *Bombax ceiba* have been reported for Hypotensive and Hypoglyceamic, analgesic, antioxidant, antiinflamatory, hepatoprotective, immunomodulatory, antimicrobial activities.[10,11]

Samanea saman (Jacq.) is locally known as 'Rendi'or'Fulkoroi. The plant is used traditionally in diarrhea, intestinal diseases, stomach ache, colds and headache.[12] The leaves were reported to contain tannins, flavonoids, steroids, saponins, cardiac glycosides are terpenoids.[12]

MATERIALS AND METHODS

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), potassium ferricyanide, potassium acetate, phosphate buffer, catechin (CA), ferrous ammonium sulphate, butylated hydroxytoluene (BHT), gallic acid (GA), ascorbic acid (AA), AlCl3, Trichloro acetic acid (TCA), sodium phosphate, ammonium molybdate, tannic acid, quercetin (QU), EDTA, thiobarbituric acid (TBA), acetyl acetone and FeCl3 were purchased from Sigma Chemical Co.(St.Louis,MO,USA); vanillin was obtained from BDH; Folin-Ciocalteus's phenol reagent and sodium carbonate were obtained from Merck (Damstadt, Germany).

Collection of plant materials

Bark of *Syzygium cumini* (SCB) was collected in October, 2015 and flower and fruits from *Samanea saman* (SSF) and *Bombax ceiba* (BCF) respectively were collected in January 2016 from the area of Rajshahi University Campus, Rajshahi, Bangladesh. All plants materials were then washed with fresh water to remove dirty materials and were shade dried for several days with occasional sun drying. The dried materials were ground into coarse powder by grinding machine, and the materials were stored at room temperature for future use.

Preparation of extracts

About 300 gm of all dried powdered plants materials were taken in amber colored extraction bottles (2.5 liter capacity). Methanol was used to prepare plants extracts. All plants materials were soaked with methanol (500mL × 3 times). The sealed bottles were kept for 7 days with occasional shaking and stirring. The extracts were filtered through cotton and then Whatman No.1 filter papers and then the extracts were concentrated with a rotary evaporator under reduced pressure at 45°C to afford crude bark extract. The three different crude extracts from three different plants parts were than stored at 4°c for further analysis.

Method for phytochemical analysis

Photochemical compositions of the all extracts were determined through standard analytical technique where it was just assayed to detect the presence of phytochemicals. Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids, carbohydrates, saponins, reducing sugar, tannins and terpenoids were carried out for the extract by the method described previously.

Method for quantitative analysis of phytochemicals
Determination of total phenolics

The TPC of extracts were determined using the Folin-Ciocalteu reagent (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) as previously described by Kim et al.[13] The total phenolics were quantified using a calibration curve (standard curve equation: $y = 0.00202x + 0.0036$, $R2 = 0.9994$) constructed from measurements of the standard gallic acid concentrations and expressed as mg gallic acid equivalent (GAE) per mg of dry extract weight.

Determination of total flavonols

Total flavonols were estimated as quercetin equivalents, expressed as mg of quercetin per gm of dry extract by the method of Miliauskas et al.[14] The quercetin calibration curve (standard curve equation: $y = 0.0008x + 0.00337$, $R2 = 0.9942$) was prepared by mixing quercetin solution with 2 ml (20 gm/l) AlCl3 and 6 ml (50 gm/l) sodium acetate. The absorption at 440 nm was read after 2.5 h at 20ºC. The same procedure was carried out with 2 ml of plants extracts (10 gm/l) instead of quercetin solution.

Determination of total flavonoids

Total flavonoids were estimated using the method described by Ordonez et al.[15] with some modification. To 0.5 ml of samples/standard, 1.5 ml of methanol, 100μl of 10% aluminum chloride, 100μl of 1M potassium acetate solution and 2.8 ml of distilled water was added. After one hour 30 minutes of incubation at room temperature (RT), the absorbance was measured at 420 nm. Total flavonoid contents were expressed in terms of catechin equivalent, CAE (standard curve equation: $y = 0.0041x + 0.0335$, $R2 = 0.9884$), mg of CA/g of dry extract.

Determination of total proanthocyanidins

Determination of proanthocyanidins was based on the procedure reported by Sun et al.[16] A volume of 0.5 ml of 0.1 mg/mL of extracts/standard solution was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml hydrochloric acid; the mixture was allowed to stand for 15 minutes. The absorbance was measured at 500 nm. Total content of proanthocyanidins was expressed in terms of catechin equivalent, CAE (standard curve equation: $y = 0.0157x + 0.014$, $R2 = 0.9985$), mg of CA/g of dry extract.

Determination of antioxidant activity
Determination of total antioxidant capacity

Total antioxidant capacity (TAC) of samples/standard was determined by the method reported by Prieto et al.[17] with slight modifications. 0.5 ml of samples/standard at different concentrations was mixed with 3 ml of reaction mixture containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 1% ammonium molybdate into the test tubes. The test tubes were incubated at 95°C for 10 minutes to complete the reaction. The absorbance was measured at 695 nm using a spectrophotometer against blank after cooling at RT. Ascorbic Acid was used as

reference standard. Increased absorbance of the reaction mixture indicated increase total antioxidant capacity.

Determination of ferrous reducing antioxidant capacity

The ferrous reducing antioxidant capacity of samples/ standard was evaluated by the method described by Oyaizu et al.[18] 0.25 mL of extracts and ascorbic acid as standard at different concentration were mixed with potassium buffer (.625ml, 0.2 M, pH 6.7) and 0.625 ml of 1% potassium ferricyanide [K3Fe(CN).[6] The mixture was incubated at 50◦C for 20 min. After incubation, 0.625 ml of 10% trichloroacetic acid (TCA) solution was added to each tube and the mixture was centrifuged at 3000 rpm for 10 min. Clear supernatant (1.8 ml) was mixed with equal amount of distilled water, 0.36 ml of ferric chloride (0.1% w/v) solution was added and absorbance was recorded at 700 nm. Reducing capacity of the extracts was linearly proportional to the concentration of a sample.

DPPH free radical scavenging assay

DPPH free radical scavenging activity was determined spectrophotometrically as described by Cheel et al,[19] Free radical scavenging activity of each sample was calculated by using following formula:

DPPH Radical scavenging rate (%) = [{A0 − A}/ A0] × 100

Where A0 (control) was the absorbance of DPPH blank solution, and A was the final absorbance of the tested sample after 30 min of incubation.

ABTS radical scavenging assay

The ABTS method was used according to Re et al.[20] The scavenging rate was calculated using the formula:
ABTS Radical scavenging rate (%) = [{A0 − A}/ A0] × 100

Where A0 (control) was the absorbance of ABTS blank solution, and A was the final absorbance of the tested sample after 6 min of incubation.

Nitric oxide radical scavenging activity

NO reacts with oxygen to produce stable products (nitrate and nitrite), the quantities of which can be determined using Griess reagent procedure described by Marcocci et al.[21] 2 mL of 10 mM sodium nitroprusside dissolved in 0.5 mL phosphate buffer saline (pH 7.4) is mixed with 0.5 mL of sample at various concentrations. The mixture is then incubated at 25ºC. After 150 min of incubation, the incubated mixtures were then mixed with 0.5 mL of Griess reagent. The mixture is then incubated at room temperature for 30 min and its absorbance pouring into a cuvette is measured at 546 nm. Catechin was used as reference standard. The amount of nitric oxide radical inhibition is calculated following this equation:
% inhibition of NO radical = [{A0 − A}/ A0] × 100

Where A0 (control) was the absorbance of the NO radical solution without test sample and A is the absorbance of the test sample.

Superoxide scavenging assay

The assay was done following the method of Khanna.[22] 0.1mL each of the plant extracts and standard were added to the reaction mixture containing 50mM phosphate buffer (pH-7.6), 20µg/ml riboflavin, 12mM EDTA and 0.1mM NBT. The reaction was initiated by illuminating the reaction mixture for 5 minutes and the absorbance was measured at 590nm. Qurecitin was used as reference standard. The % of nitric oxide radical inhibition is calculated following this equation:

Superoxide scavenging assay (%) = [{A0–A}/ A0] × 100

Where A0 was the absorbance of the control, and A is the absorbance of the extractives/standard.

Lipid peroxidation inhibition assay

Malondialdehyde content was estimated according to the methods described by unni et al.[23] 15% w/v Trichloroacetic acid, 0.375%w/v thiobarbituric acid and 0.25N Hydrochloric acid were mixed to form the stock Thio-barbituric acid (TBA) – Tricholoro acetic acid (TCA) – HCl reagent. Albino rats (180-200g) of either sex were used for the study. After decapitation, the brain was removed carefully. The tissue was immediately weighed and homogenated with cold 1.15%w/v KCl to make 10%v/v homogenate. The homogenate (0.5ml) was added to 1 ml of various concentrations of the extracts. Then the mixture was incubated for 30 min. The per-oxidation was terminated by the addition of 2 ml of TBA-TCA –HCl reagent. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the supernatant was measured at 535 nm. Qurecitin was used as reference standard. The % inhibition of various concentrations was calculated by using the formula.
Lipid peroxidation assay (%) = [{A0 − A}/ A0] × 100

Where A0 is the absorbance of the control, and A is the absorbance of the extractives/standard.

Brine Shrimp lethality bioassay

Brine shrimp lethality bioassay was carried out according to the method described by Persoone[24] for the determination of cytotoxic property of the sample extracts.

In vitro anti-diabetic activity (α-Amylase inhibition test)
3, 5-Dinitrosalicylic acid method (DNSA)

The inhibition assay was performed according to Miller[25] using DNSA method. 500µl of extracts at different concentration were added to 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) containing .5mg/ml of α-amylase

solution and were incubated at 37°C for 10 min, followed by addition of 500 μL of 1% starch solution in 0.02 M sodium phosphate buffer (pH6.9) all the test tubes. The reaction was stopped with 1.0 ml of 3, 5 DNSA reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. The control samples were also prepared accordingly without any plant extracts.The results were expressed as % inhibition calculated using the formula:

α-amylase inhibition activity = $[1 - \{(A1-A2)/A0\}] \times 100$

Where A1 is the absorbance of test sample, A2 is the absorbance of product control (sample without α-Amylase solution) and A0 is the absorbance of negative control (α-Amylase without extract).

Starch-iodine Colour Assay

Screenings for α-amylase inhibition by extracts were carried out according to Xiao et al.[26] with slight modification based on the starch-iodine test. 500μl of extracts at different concentration were added to 500 μL of 0.02 M sodium phosphate buffer (pH6.9 containing 6 mM sodium chloride) containing .5mg/ml of α-amylase solution and were incubated at 37°C for 10 min. then 500 μL soluble starch (1%, w/v) was added to each reaction test tube and incubated at 37°C for 15 min. 1 M HCl (20 μL) was added to stop the enzymatic reaction, followed by the addition of 100 μL of iodine reagent (5 mM I2 and 5 mM KI). The color change was noted and the absorbance was read at 620 nm. The results were expressed as % inhibition calculated using the formula:

α-amylase inhibition activity = $[1 - \{(A1-A2)/A0\}] \times 100$

Where A1 is the absorbance of test sample, A2 is the absorbance of product control (sample without α-Amylase solution) and A0 is the absorbance of negative control (α-Amylase without extract).

Anti-inflammatory bioassay *in vitro* (Inhibition of protein denaturation method)
Egg Albumin denaturation inhibition method

According to previously reported protocol,[27] The reaction mixture consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (pH 6.4) and 2 ml of varying concentrations of the test extract. Similar volume of double-distilled water served as control. Then the mixtures were incubated at 37°C ± 2°C for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm. Aspirin was used as reference drugs and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition = $100 \times ([Vt/Vc] - 1)$.

Where, Vt = absorbance of test sample, Vc = absorbance of control.

Inhibition of Bovine serum albumin (BSA) denaturation method

Inhibition of protein denaturation was evaluated by the method of Sakat et al.[28] with slight modification. 500 μL of 1% bovine serum albumin was added to 100 μL of plant extract. This mixture was kept at room temperature for 10 minutes, followed by heating at 51°C for 20 minutes. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Acetyl salicylic acid was taken as a positive control. The experiment was carried out in triplicates and percent inhibition for protein denaturation was calculated using:

% Inhibition = $100 - [(A1-A2)/A0 \times 100]$

Where A1 is the absorbance of the sample, A2 is the absorbance of the product control and A0 is the absorbance of the positive control.

RESULTS AND DISCUSSION

Qualitative phytochemical screening

Plant secondary metabolites including tannins, glycosides, saponins and flavonoids are responsible for tremendous pharmacological activities for instance antimicrobial, antioxidant, antifungal and anticancer which may benefit in protection against chronic diseases.[29,30] Table 1, illustrates the qualitative analysis of different classes of phytochemical in different plants extracts. Tannins, steroids, flavonoid, saponins, cardiac glycosides, anthracenosides, alkaloids, Coumarins and triterpenoids were found to be the major secondary metabolites present in the extracts. Alkaloids were detected in all tested extracts except SCB. Coumarins were detected in SCB while absent in BCF and SSF. Tannins did not make their presence in SCB. Presence of saponins was confirmed only in SCB.

Total phenolic, flavonoids, flavonols and proanthocyanidin contents

Polyphenols, such as flavonoids, phyenylpropanoids, phenolic acids, etc. are responsible for the free radical scavenging and antioxidant activities of plants. Various studies confirm that flavonoid groups exhibit high potential biological activities such as antioxidant, antiinflammatory and anti-allergic reactions.[31–32]

Table 2 showed the results of total phenolic content of different plants extracts. Total phenol and total flavonoid contents of the extracts were expressed in gallic acid and catechin equivalents respectively. SCB extract possess the heights content of phenol to compare with another extract. On the other hand both of the extracts have moderate flavonoids value, among of them SCB has significant ($P<0.05$) higher flavonoids value. Presence of high concentration of phenolic compounds causes the high percentage scavenging value of the extracts. Dietary flavonols and proanthocyanidins in particular offer significant cardiovascular health benefits.[33] Proanthocyanidin rich extract has preventive actions on diseases, such as atherosclerosis, gastric ulcer, large bowel cancer, cataracts, diabetes and inflammation.[34]

Total flavonol and total proanthocyanidin contents of the extracts were expressed in catechin and quercetin equivalents respectively. BCF rich in flavonol and the value was 85.75±7.07 mg/g catechin equivalent, whereas flavonol content of SSF was not determined. The total proanthocyanidin contents of all extracts were in the following order SCB>SSF>BCF. The phenolic and flavonoids content of the extracts under this investigation correlates ($P<0.01$) with their antioxidant activity. Our findings strongly suggest that the polyphnols are important components of these plants and some of its pharmacological effects like anti-inflammatory and antidiabetic activity could be attributed to the presence of these valuable constituents.

Total antioxidant capacity

All the extracts showed a good total antioxidant activity, which was concentration-dependent (figure-1A). The antioxidant capacity may be attributed to their chemical composition and phenolic content. Total antioxidant capacity of the extracts and standard recorded at the highest dose of 200 µg/ml was in order of AA > SCB > BCF > SSF. The current analysis reveals that SCB and BCF displayed the uppermost antioxidant capacity. Latest researches proved that flavonoids and related polyphenols contribute substantially to the phosphomolybdate quenching capability of medicinal plants.[35] SCB and BCF exhibited the highest antioxidant index comparable with SSF.

Ferrous reducing antioxidant capacity

The reducing power of a compound could be used as an indicator of its potential antioxidant capacity, and the ability to reduce Fe^{3+} to Fe^{2+} is often assayed as an indicator of electron-donating activity.[18] As evidenced in Figure 1, all the extracts, as well as AA, presented linearly dose-dependent increases in absorbance, and their reducing power followed the order of AA>SCB>BCF>SSF. The reducing power of the extracts is probably due to the presence of phenolic compounds which might act as electron donor.[36] SCB extracts almost touch the standard curve line at highest concentration that indicates the dose dependent manner of antioxidant activity of SCB extract.

DPPH radical scavenging activity

Each extracts were measured for their ability to scavenge DPPH free radicals and results showed on table 3. The scavenging effects of extracts at concentration 1000 µg/mL were as follows: SCB > SSF > BCF and at this concentration the inhibition activity of the all extracts were almost identical to that of the standard AA activity. The lowest IC50 value from the all extracts reflects its potent antioxidant fidelity. SCB have lowest IC50 value. DPPH radical scavenging activity of the three different plants extract showed good correlation with TPC ($P<0.05$) and TFC ($P<0.05$). Results of present investigation are attributed due to the presence of phyto-constituents most specifically; phenolic and flavonoid

are capable of donating electron and stabilizing the free radicals.[37]

ABTS radical scavenging activity

The result obtained indicated that both of the plants extract scavenge the ABTS radicals in a dose dependent pattern. Among the extracts lowest IC50 values were determined for SCB (8.53±2.04µg/ml) while highest IC50 values were recorded for SSF (24.95±5.51µg/ml) as shown in Table 3. However IC50 values of SCB were almost similar that of the ascorbic acid (7.40±1.03µg/ml). IC50 value of SSF were significantly ($P<0.05$) different from SCB. The ABTS scavenging activity of the present study suggests that the phyto-constituents within the extracts donate electron/hydrogen while minimizing the oxidative stress.[38] Furthermore correlation analysis indicated significant correlation between ABTS radical scavenging activity and TPC ($P<0.05$) as well as TFC ($P<0.05$).

Lipid peroxidation inhibition activity

The scavenging activity in lipid peroxidation inhibition were in the order of SCB > SSF > BCF. IC50 values (317.2±2.981µg/ml) showed by SCB were significantly ($P<0.05$) lower as compared to BCF values (356.5±6.62µg/ml). In this study the *in vitro* ability of plants extract to prevent the production of TBARS depicts the potential of samples to inhibit oxidation in lipid system. Significant correlation was observed with TPC ($P<0.05$) and TFC ($P<0.05$). Phenolic compounds are very important plant constituents because they exhibit antioxidant activities by inactivating lipid free radicals, or by preventing the decomposition of hydro-peroxides into free radicals.[39]

Superoxide Anion Radical Scavenging Activity

Although superoxide anion is a weak oxidant, but it lead to the generation of powerful and hazardous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress. Therefore, it is very important to study the scavenging of superoxide anion.[40] All the extracts possess moderate Superoxide Anion radical scavenging activity with lowest IC50 values indicate that pharmaceuticals important of these plants. The IC50 (table 3) values in superoxide scavenging activities were in the order of SCB > SSF > BCF. When compared to quercetin, the superoxide scavenging activity of the SCB was found to be statistically ($P<0.05$) similar. A significant correlation was detected with TPC ($P<0.05$) and TFC ($P<0.05$). This strong superoxide radical neutralizing capacity of the all plants might be functional therapeutically against oxidative stress induced ailments

NO radical scavenging activity

NO itself is not a very reactive free radical, however, over production of NO is responsible for initiating lipid peroxidation and production of free radicals.[41] Therefore, it is very important to study the scavenging of NO radicals. All the extracts possess moderate NO

radical scavenging activity indicate pharmaceuticals important of these plants. The IC50 (table 3) values in NO radical scavenging activity were in the order of SCB > SSF > BCF. The potent scavenging ability of the all plants extract might be due its bioactive phytoconstituents, which are able to minimize the oxidation of biological macromolecules. A significant correlation was detected with TPC (P <0.05) and TFC (P>0.05).

Brine Shrimp cytotoxic ability

The toxicity of all the crude extracts to brine shrimp was determined on *A. salina*.[42] The extracts were found to show significant activity against brine shrimp nauplii. In this bioassay, the mortality rate of brine shrimp was found to be increased with the increase in concentration of the test sample. So it was observed that there is a positive correlation between brine shrimp toxicity and cytotoxicity. The median lethal concentration (LC50) of brine shrimp lethality was found to 54.33, 207.75, 326.08 and 6.25μg/mL from SCB, BCF, SSF and Gallic Acid respectively which was obtained from probit statistical analysis. The data and result of the test compound and LC50 of standard gallic acid were given in the (Table 4). High value of LC50, indicates the low toxic effect of SSF. Whereas SCB showed comparatively low LC50 indicate its potent toxicity. Standard cytotoxic agent gallic acid was found to exhibit higher cytotoxicity giving lower LC50 values of 6.25 μg/mL.

α- amylase inhibitory activity

The polyphenolic compounds in plants inhibit the activities of carbohydrate hydrolyzing enzymes, such as α -amylase and α -glucosidase, because of their ability to bind with proteins.[43] As alternative antidiabetic drugs, several α -amylase and α -glucosidase inhibitors have recently been isolated from medicinal plants.[44] α-amylase inhibitory activities of the all plants extract illustrated in Figure- 3A and 3B. Moreover when treated with at concentration 1000μg/ml in 3, 5-Dinitrosalicylic acid (DNSA) method, SCB extract showed the highest inhibitory activity (53.95%) among three plant extracts. SSF caused second highest inhibition indicate its antidiabetic potency with inhibitory value 49.63%. On the other hand BCF have lowest potency but almost similar to that of SSF. At 1000μg/mL concentration 41.78, 33.50 and 34.83 percentage of α-Amylase inhibition were observed in Starch iodine color assay method by SCB, SSF and BCF respectively, where standard Acarbose exhibit 57.70 percentage inhibitions. A significant correlation was detected with TPC (P <0.05) and correlation (P<0.05) was observed with TFC. These studies suggest that SCB possess highest anti-diabetic properties which might be used to prepare pharmaceutical agents for hyperglycemic treatment.

***In vitro* anti-inflammatory activity**

It was already proved that conventional NSAID's drug use for the treatment of inflammatory diseases do not act only by the inhibition of endogenous prostaglandins production but also by prevention of denaturation of proteins.[45] Thus anti-denaturation assay is the convenient method to check the anti-inflammatory activity. In the present study for *in vitro* anti-inflammatory test, the BCF extract in egg albumin denaturation method showed mean inhibition of protein denaturation about 51.20% at doses 1000 μg/mL, whereas for Aspirin it was found to be 80.40% (Figure 3A). On the other hand SCB showed second highest inhibition activity to compare with BCF and which was significantly similar with the activity of BCF. The BCF extract showed good anti-inflammatory activity with a linear response in BSA method. Maximum inhibition of 42.11% and 75.02% was observed at 1000 μg/mL by BCF and standard anti-inflammatory drug (Aspirin) respectively. 32.89% and 28.79% inhibition were showed at the concentration of 1000 μg/mL by SCB and SSF. From the result of the present study, the extracts had shown considerable anti-inflammatory activity. The secondary metabolites like phenolic compounds and tannins which were found in preliminary phytochemical screening might be responsible for this activity.

Correlations between the biological activities and total phenolic and flavonoid contents of the different plants extracts: Table 5 shows the Correlations between the biological activities and total phenolic & flavonoid contents of the different plants extracts.

Table 1: Phytochemical constituents of three different plant extract.

Name of test		Name of sample extract		
		BCF	SSF	SCB
Alkaloids	Dragendorff's test	+	+	-
	Hager's test	+	+	-
	Wagner's test	+	+	-
	Mayer's test	+	+	-
Carbohydrate test	Benedict's test	+	+	+
	Fehling's test	+	-	+
	Molisch's test	+	+	+
Flavonoid test		+	+	+
Glycosides		+	+	+
Triterpenoids		+	-	-
Resins		-	-	-
Saponins		-	-	+
Steroids	Liebermann-Burchard's test	-	+	+
	Salkowski reaction	+	-	+
Tannins		+	+	-
Coumarins test		-	-	+
Cardio glycoside test		+	-	+
Anthracenosides test		-	-	-

Negative sign (−) indicate absence, positive sign (+) indicate presence. SCB (*Syzygium cumini* Bark), BCF (*Bombax ceiba* Fruits),and SSF (*Samanea saman* Flowers)

Table 2: Polyphenols content of three different plant extract.

Polyphenols content	SCB	BCF	SSF
Phenolics[1]	366.64 ± 17.49^a	116.47 ± 1.81^b	57.84 ± 6.66^b
Flavonoids[2]	173.71 ± 8.95^a	55.55 ± 8.34^c	78.31 ± 7.68^d
Flavonols[3]	7.05 ± 5.30^a	85.75 ± 7.07^a	N.D
Proanthocyanidins[2]	23.82 ± 3.49^a	16.14 ± 1.63^c	19.24 ± 1.67^b

NB: Each value is the average of three analyses $\pm$ standard deviation 1, 2 and 3 expressed in terms of GAE, CAE and QUE, respectively (mg of GA, CA and QU/g of dry extract, respectively). Means with superscript with different letters in the rows are significantly ($p<0.05$) different from each other.

Table 3: IC$_{50}$ values of different antioxidant activities of different plants extract.

Extracts and Standards	IC$_{50}$(μg/ml) value				
	DPPH	ABTS	Lipid Peroxidation Scavenging Assay	Superoxide radical scavenging Assay	Nitric oxide Scavenging Assay
SCB	79.25 ± 8.95^b	8.53 ± 2.04^a	317.2 ± 2.98^b	74.43 ± 4.42^a	256.3 ± 2.69^a
BCF	133.7 ± 5.75^c	12.72 ± 5.02^c	702.3 ± 6.14^c	242.8 ± 3.01^b	634.6 ± 2.11^c
SSF	246.7 ± 7.26^d	24.95 ± 5.51^b	356.5 ± 6.62^b	191.9 ± 5.28^b	492.8 ± 3.42^b
AA	55.44 ± 7.01^a	7.40 ± 1.03^a	-	-	-
QUERCETIN	-	-	248.3 ± 5.37^a	69.35 ± 3.992^a	-
CATECHIN	-	-	-	-	281.5 ± 1.86^b

Values are expressed as Mean $\pm$ SD (n= 3). Means with superscripts with different letters in the column are significantly ($P<0.05$) different from each other. The concentration which caused a half-maximal reduced radical level (IC$_{50}$) was determined. % of inhibition was plotted against concentration, and IC$_{50}$ was calculated from the nonlinear regression curve using graph pad prism software. (**-**indicate not determined), SCB (*Syzygium cumini* Bark), BCF (*Bombax ceiba* Fruits), SSF (*Samanea saman* Flowers) and AA (ascorbic acid). (Completely Randomized AOV followed by Tukey HSD All-Pairwise Compariso Test)

Table 4: Cytotoxic ability of three different plant extract.

Conc. (μg/ml)	Log conc.	% of mortality				LC$_{50}$ (μg/ml)			
		SCB	BCF	SSF	GA	SCB	BCF	SSF	GA
10	.99	20	00	10	60				
25	1.39	30	20	20	80				
50	1.69	50	20	20	90	54.33	207.75	326.08	6.25
100	1.99	60	40	40	100				
200	2.30	80	50	40	100				

Cytotoxic effect of all the extracts in brine shrimp lethality showed high value of LC$_{50}$ indicates the low cytotoxic effect.

Table 5: Correlations between the biological activities and total phenolic and flavonoid contents of the different plants extracts.

	Correlation R^2					
	TPC			TFC		
	SCB	SSF	BCF	SCB	SSF	BCF
DPPH free radical scavenging activity	.901*	.948*	.924*	.857	.861	.910*
ABTS radical scavenging activity	.924*	.997**	.860	.897*	.903*	.833
Nitric oxide scavenging activity	.991**	.970*	.950*	.999**	.999**	.940*
Superoxide scavenging activity	.921	.997**	.973*	.922	.928	.967*
Lipid peroxidation scavenging activity	.986**	.897*	.920*	.944*	.942*	.901*
α-amylase inhibitory activity	.992**	.994**	.966*	.999**	.999**	.961*
In vitro anti-inflammatory activity	.948	.974*	.786	.980*	.986*	.786

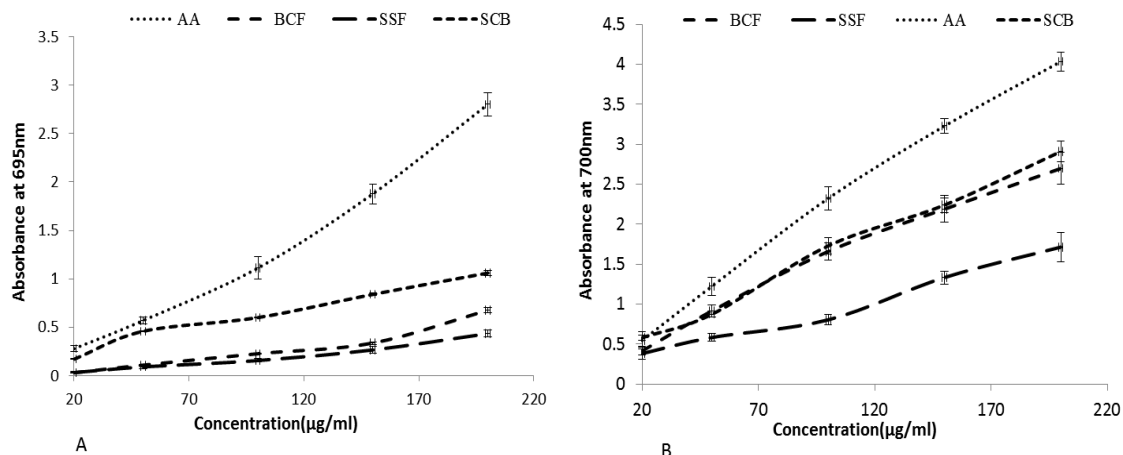*Correlation is significant at the 0.05level, ** correlation is significant at the 0.01 level.

Figure 1 (A): Total antioxidant capacity and (B) Ferrous reducing antioxidant capacity of the extract of SCB (*Syzygium cumini* Bark), BCF (*Bombax ceiba* Fruits) and SSF (*Samanea saman* Flowers). Data expressed as mean ± SD (n=3, p< .05) for all tested doses.
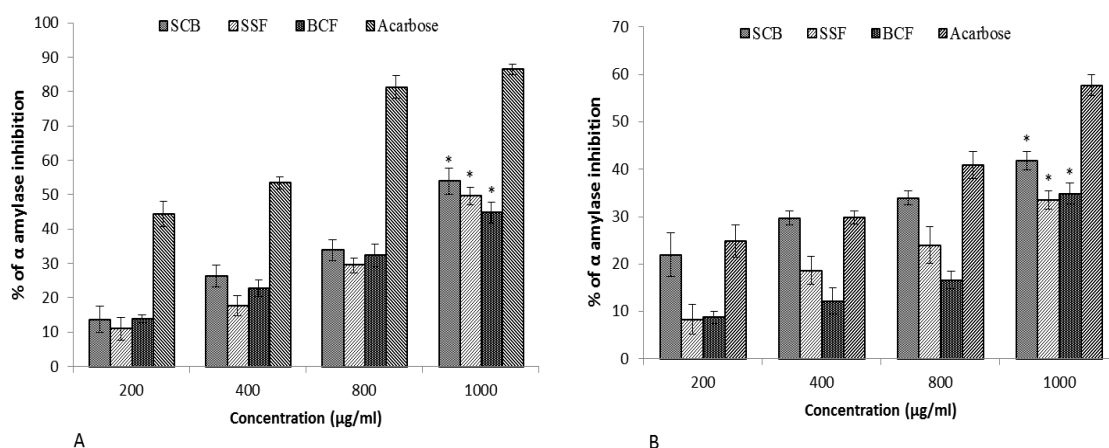

Figure 2: *In vitro* antidiabetic activity of the extract of SCB (*Syzygium cumini* Bark), BCF (*Bombax ceiba* Fruits) and SSF (*Samanea saman* Flowers) in (A) 3, 5-Dinitrosalicylic acid (DNSA) and (B) Starch-iodine Colour assay method. Data expressed as mean ± SD (n=3,*p< .01).
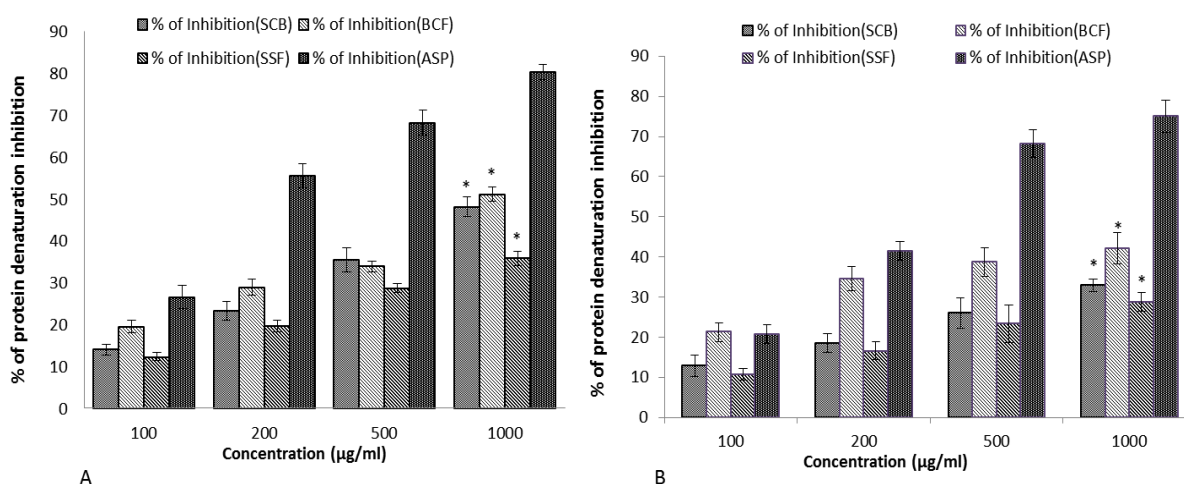

Figure 3: *In vitro* anti-inflammatory activity of the extract of SCB (*Syzygium cumini* Bark), BCF (*Bombax ceiba* Fruits) and SSF (*Samanea saman* Flowers) in (A) Egg Albumin denaturation and (B) Bovine serum albumin (BSA) denaturation inhibition method. Data expressed as mean ± SD (n=3,*p<0.01).

ABBREVIATIONS

ABTS, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid; SCB, *Syzygium cumini* Bark; BCF, *Bombax ceiba* Fruits; SSF, *Samanea saman* Flowers; AA, Ascorbic acid; DPPH; 2,2-Diphenyl-1-Picrylhydrazyl; EDTA, ethylene diamine tetra acetic acid; PBS, phosphate buffer saline; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; TFC, total flavonoid content; TPC, total phenolic content; QU, Quercetin; CAT, Catechin; GA, Gallic acid; OS, Oxidative stress; ROS: Reactiveoxygen species; NO, Nitricoxide; LC, Lethal concentration; IC, Inhibitory concentration.

CONCLUSION

In the present investigation it was observed that different plants parts varied in their phytochemical constituents. The high phenolic content was positively correlated with free radical scavenging activity of the all extracts. These results were supported by quantitative assays as well as qualitative screening by using analytical profiling. Although methanol extract of dry bark from *Syzygium cumini* showed maximum phenolic content as observed through TPC, TFC determination and ABTS, DPPH, NO, Superoxide radical scavenging activity was also exceptionally high in methanolic extract of *Syzygium cumini* bark. These findings imply potent antioxidant ability of *Syzygium cumini* bark, on the other hand *Bombax ceiba* fruits *and Samanea saman* flower showed their moderate andioxidant activity. *In vitro* anti-diabetic and anti-inflammatory activity were assayed using α-amylase inhibition and protein denaturation inhibition method, where high percent value of inhibition by *Samanea saman* bark and *Bombax ceiba* fruits indicate their potent antidiabetic and anti-inflammatory ability respectively. This study demonstrated the potency of crude extracts of the all plants parts as significant source of natural phytoconstituents and antioxidant supplements, indicating their strong potential to be used in traditional medicine system.

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