

In vitro Biochemical Assessments of Methanol Stem Bark Extracts of *Ficus sycomorus* Plant

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Abstract

Natural antioxidants are important in disease prevention and promotion of health. We determined antioxidants' activity using DPPH radical scavenging activity, Hydrogen peroxide radical scavenging activity and Ferric reducing antioxidants power. The extracts significantly ($p < 0.05$) exhibited strong antioxidants activities at concentrations (20, 40, 60, 80 and 100 mg/ml) compared to L-Ascorbic. Preliminary phytochemical screening showed the presence of tannins, saponins, flavonoids, terpenoids, phenols and steroids while glycoside and proteins were absent. The extracts significantly ($p < 0.05$) exhibited a receptor binding affinity when estimated using hemolytic inhibition assay and hemagglutination inhibition assay at 20, 40, 60, 80 and 100 mg/ml on human erythrocytes. Antibacterial activity (% Inhibition) was tested against five pathogenic organisms (*E. coli*, *S. aureus*, *S. typhi*, *B. cereus* and *P. aeruginosa*). The extracts significantly ($p < 0.05$) inhibited *E. coli* (30.82 ± 8.73) at 2 mg/ml, *S. aureus* (30.23 ± 6.56 , 40.31 ± 2.88 and 43.38 ± 0.94) and *S. typhi* (30.49 ± 0.81 , 36.99 ± 0.50 and 47.69 ± 1.02) at 2 mg/ml, 4 mg/ml and 6 mg/ml, respectively, and *P. aeruginosa* (41.82 ± 1.12 , 49.02 ± 0.34 , 56.03 ± 0.50 , 69.90 ± 0.27 and 73.26 ± 0.43) at 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml and 10 mg/ml compared to standard drug. The standard drug significantly ($p < 0.05$) inhibited *B. cereus* (28.82 ± 0.80 , 40.40 ± 0.40 , 44.92 ± 1.20 , 56.20 ± 2.52 and 62.72 ± 0.79) at 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml and 10 mg/ml compared to the stem bark extracts.

Keywords: : Antioxidant activity, Antibacterial activity, Antihemolytic assay, *Ficus sycomorus*

1. Introduction

Antioxidants are substances that protect living cells from the damage caused by unstable molecules known as free radicals. Antioxidants are known to interact and stabilize free radicals thereby preventing damage. The free radical damage may lead to the development of cancer (Prior *et al.*, 2005). Antioxidant molecules are capable of slowing or preventing the oxidation of other molecules. Oxidation refers to the chemical reaction that transfers electrons from one substance to another. Oxidation reactions produce free radicals which start chain reactions that damage the cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibiting other oxidation reactions. Examples of some antioxidants are Beta-carotene, lycopene, vitamins A, C and E (Lopez *et al.*, 2007).

One major role of antioxidants is protecting the living cells from potentially damaging oxidative stress resulting from imbalance between the formation of Reactive Oxygen Species (ROS) and the body antioxidant defense. Naturally occurring antioxidants are used in

foods because of their potential in health promotion, disease prevention, high safety and consumer acceptability. Antioxidants are used in food industry to prevent deterioration, nutritional losses and off-flavoring in various foods, especially those containing polyunsaturated fatty acids (Gorinstein *et al.*, 2003).

Ficus sycomorus Linn belongs to the family of *Moraceae*, comprising about 40 genera and over 1,400 species of trees, shrubs, vine and herbs, often with milky latex juices. The plant grows up to 20 m with widely spreading branches and crown. *F. sycomorus* fruits, stem bark and root are widely used in Nigeria, Niger, Mali, South Africa, Guinea, Kenya, Tanzania, Somalia, Ethiopia and Ivory Coast for the treatment of various diseases such as cough, diarrhea, skin infections, stomach disorders, liver disease, epilepsy, tuberculosis, lactation disorders, helminthiasis, infertility, sterility and diabetes mellitus (Igbokwe *et al.*, 2010; Adoum *et al.*, 2012). The plant has also been reported to be a potent antimicrobial agent against ciprofloxacin resistant *Salmonella typhi* (Adeshina *et al.*, 2010). *F. sycomorus* extracts have been reported by Auda (2012) for the treatment of various skin diseases while the decoction is used for treating

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gastrointestinal tract problems. It is further used as seasoning; the stem bark is dried and added to cake as a condiment, eaten raw or cooked as soup. Nkafamiya *et al.* (2010) reported that the proximate analysis of *F. sycomorus* plant showed high contents of proteins and crude fibers while ash, lipid and carbohydrate contents were within the range expected for dry leafy vegetables. In view of the above background, the present study seeks to investigate the antioxidant activities, hemolytic inhibition assay, hemagglutination inhibition assay and antibacterial activities of methanol stem bark extract of *F. sycomorus*

2. Materials and Methods

2.1. Collection of Plant Material

Fresh stem bark of *F. sycomorus* plant was collected from around Sangere, Girei Local Government Area, Adamawa State. Sangere is located on latitude 9° 11' 15" N and longitude 12° 20' 29" E, on the North bank of River Benue. The plant was taxonomically identified and authenticated in the Plant Science Department of Modibbo Adama University of Technology, Yola. The stem bark was air dried in the laboratory for 7 day and thereafter made into powder using electric blender. The coarse material was sieved using 0.3 mm Endicott test sieve.

2.2. Preparation of the Plant Extract

Air dried and powdered plant material 300 g was extracted with methanol by cold extraction process for 24 h with intermittent stirring. The solvent extract was filtered using a sterilized Whatman filter paper No.1 to obtain a particle free extract. The solvent extract was concentrated by evaporation of the solvent at < 50°C using rotary evaporator and vacuum oven to obtain dry powder. The extract was stored until use.

2.3. Qualitative Phytochemical Screening

Qualitative phytochemical screening of the freshly prepared crude extract was tested for the presence of carbohydrates, alkaloids, flavonoids, steroids, phenols, tannins, saponins, glycosides and proteins as described by Nweze *et al.* (2004) and Senthilkumar and Reetha, (2009).

2.4. Determination of DPPH (2, 2-diphenyl-2-picrylhydrazyl) Radical Scavenging Activity

The DPPH radical scavenging capacity of the plant extracts was determined according to the method described by Sasidharan *et al.* (2007). The free radical scavenging activity of the extract was measured by the decrease in absorbance of methanol solution of DPPH. The DPPH radical scavenging method was used for the determination of the antioxidant capacity of the extracts. A different concentration of the plant extracts (20, 40, 60, 80 and 100 mg /ml, in methanol) was added at an equal volume (10 ml) to methanol solution of DPPH (400 µg/ml). A different concentration of L-Ascorbic acid (20, 40, 60, 80 and 100 mg /ml) was used as the standard antioxidant. The antioxidant activity of the stem bark extract was compared with L-Ascorbic acid. IC₅₀ values (where 50 % of the radicals were scavenged by the test

sample) were interpolated from the reference inhibition curve. After 30 min incubation at room temperature, the absorbance values were measured at 517 nm on a spectrophotometer (VIS 721, PEC MEDICAL USA) and converted into the percentage antioxidant activity using the following equation:

$$\text{DPPH antiradical scavenging capacity (\%)} = \frac{\text{Absorbance of sample} - \text{Absorbance of blank}}{\text{Absorbance of blank}} \times 100$$

2.5. Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide scavenging activity was determined using the method of Repon *et al.* (2013). A solution of hydrogen peroxide (40 mmol/l) was prepared in phosphate buffer (50 mmol/l, pH 7.4). The concentration of hydrogen peroxide was determined by absorbing at 230 nm using a spectrophotometer. Extract (20-100 mg/ml) in phosphate buffer was added to hydrogen peroxide and absorbance at 230 nm was determined after 10 minutes against a blank solution containing a phosphate buffer without hydrogen peroxide. L-Ascorbic acid was used for comparison. The percentage of hydrogen peroxide scavenging was calculated using the following:

$$\text{(\% of H}_2\text{O}_2\text{ scavenging activity)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

while Ascorbic acid was used as a positive control.

2.6. Ferric Reducing Antioxidant Power (FRAP assay)

In ferric reducing antioxidant power assay, various concentrations (20, 40, 60, 80 and 100 mg/ml) of the methanol extracts were mixed with 1 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide in separate test tubes. The reaction mixtures were incubated in a temperature controlled water bath at 50°C for 20 minutes followed by the addition of 1 ml of 10% trichloroacetic acid. The mixtures were then centrifuged for 10 minutes at room temperature. The supernatant obtained (1 ml) was added with 1 ml of deionized water and 200 µl of 0.1% FeCl₃. The control was prepared in the same manner as the samples except that 1% potassium ferricyanide was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm. L-ascorbic acid was used as a standard. The reducing power was expressed as an increase in A_{700 nm} after blank subtracted (Bancrejee *et al.*, 2008). Percentage inhibitory activity was calculated using the following:

$$\text{(\% Inhibitory activity)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

while Ascorbic acid was used as a standard.

2.7. Anti-hemolytic Assay

Inhibition of H₂O₂ induced red blood cell hemolysis of methanol extract was determined as described by Tavazzi *et al.* (2001). The erythrocytes, from human blood, was separated by centrifugation and washed with saline or isotonic sodium phosphate buffer (pH 7.4) until the supernatant becomes colorless. The erythrocytes were then diluted with saline or phosphate buffer to give a 4%

suspension. Varying amounts of the extract (20, 40, 60, 80 and 100 mg/ml), with saline or buffer, was added to 2 ml of the suspension of erythrocytes and the volume was made up to 3.5 ml with saline or buffer. This mixture was pre-incubated for 120 min and then 0.5 ml H₂O₂ solutions of appropriate concentration in saline or buffer was added. The concentration of H₂O₂ in the reaction mixture was adjusted so as to bring 90% hemolysis of blood cells after 120 min incubation. Incubation was being concluded after these time intervals by centrifugation during 5 min at ×1000 g and the extent of hemolysis was determined by the measurement of the absorbance at 540 nm corresponding to hemoglobin liberation. The anti-hemolytic activity was expressed as the inhibition percentage and was calculated using the following formula:

$$\text{Anti-hemolytic activity (\%)} = \frac{\text{Control 540 nm} - \text{Sample 540 nm}}{\text{Control 540 nm}} \times 100$$

where, Sample_{540 nm} was the absorbance of the sample and Control_{540 nm} was the absorbance of the control.

2.8. Hemagglutination Inhibition Assay

The hemagglutination activity of the methanol stem bark extracts of *F. sycomorus* plant was tested against human erythrocyte blood groups A⁺, B⁺, AB⁺ and O⁺ as described by Saha *et al.* (2009). Stock solution of the test samples was prepared at concentration of 20, 40, 60, 80 and 100 mg/ml and each solution was serially diluted. Fresh blood from healthy volunteers was collected, centrifuged and the erythrocytes were separated. 4% erythrocyte suspension was prepared in phosphate buffer (pH 7.4) for all blood groups. One ml of the extract dilution was taken with 1 ml of 4% erythrocyte and incubated at 4 °C. After incubation, the results were noted. Smooth formation in the bottom indicated a negative activity, while a rough granular deposition at bottom showed a positive activity. The intensity of hemagglutination was determined from the extent of deposition.

2.9. Antibacterial Activity

The antibacterial activity of the methanol stem bark extracts of *F. sycomorus* plant was determined as described by Akinyemi *et al.* (2005) using the modified broth dilution technique method. Five test tubes were dispensed with 2 ml of sterile Nutrient broth followed by addition of 0.1ml of standardized inoculums of test organisms (*E. coli*, *S. aureus*, *S. typhi*, *B. cereus* and *P. aeruginosa*) to each test tube. Various concentrations (2, 4, 6, 8 and 10 mg/ml) of the stem bark extracts were added, and the test tubes were incubated aerobically at 37°C for 18-24 hr. Two control tubes were then maintained for each batch. These include the antibiotic control (antibiotic, growth medium and organism) and organism control (growth medium only and test organism). The antibacterial activity of the *F. sycomorus* extracts, antibiotic control and organism control were read using a colorimeter at 490 nm. Percentage growth inhibition was computed using the following given formula:

$$\text{Percentage growth Inhibition (C)} = 100 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.10. Statistical Analysis

Results are reported as mean ± SD. The difference between two means was analyzed using one-way analysis of variance (ANOVA). Significance was taken at p < 0.05. SPSS version 20 (USA) was used for the analysis.

3. Results

3.1. Phytochemical Screening

The preliminary phytochemical screening of *F. sycomorus* stem bark extract (Table 1) showed the presence of tannins, saponins, flavonoids, glycosides, proteins, phenols terpenoids and steroids while glycosides and proteins were absent.

Table 1. Phytochemical composition of methanolic stem bark extracts of *F. sycomorus* plant

Phytochemicals	<i>F. sycomorus</i> stem bark
Tannins	+
Saponins	+
Terpenoids	+
Flavonoids	+
Glycosides	-
Proteins	-
Phenols	+
Steroids	+

Key: Present: +, Absent: -

3.2. Determination of DPPH Radical Scavenging Activity

Figure 1 shows the result of the DPPH radical scavenging activity of *F. sycomorus* stem bark extract. The result shows that the stem bark extracts significantly (p < 0.05) exhibited a radical scavenging activity compared to L-Ascorbic acid. The half maximum inhibitory concentration (IC₅₀) of the stem bark extract and L-Ascorbic acid were interpolated graphically and found to be 24.02 mg/ml and 20.00 mg/ml, respectively.

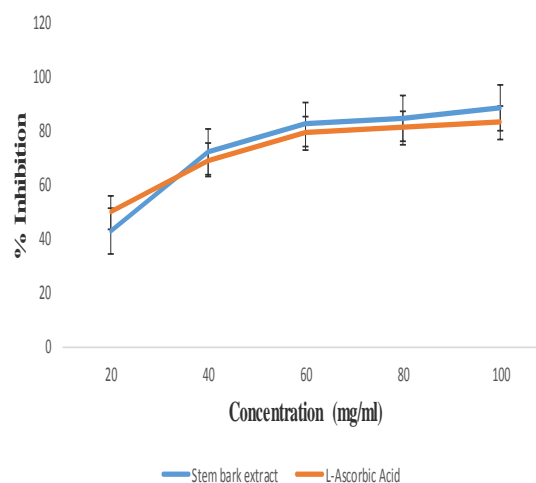


Figure 1: DPPH radical scavenging activity of methanol stem bark extract of *F. sycomorus* plant. Each values is expressed as mean ± SD, n = 3, (p < 0.05).

3.3. Hydrogen Peroxide Radical Scavenging Activity

The hydrogen peroxide radical scavenging activity of the stem bark extract was measured in comparison with L-ascorbic acid (Figure 2). Results showed that the stem bark extract significantly ($p < 0.05$) exhibited an antioxidant radical scavenging activity compared to the L-ascorbic acid.

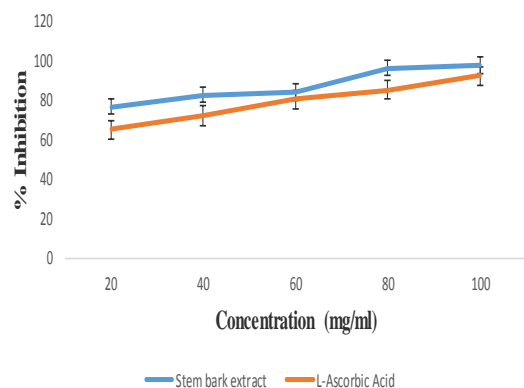


Figure 2: Hydrogen peroxide scavenging activity of methanol stem bark extract of *F. sycomorus* plant. Each value is expressed as mean \pm SD, $n = 3$, ($p < 0.05$)

3.4. Ferric Reducing Antioxidant Power

The antioxidant activity determined using Ferric reducing antioxidants power showed that the stem bark extract significantly ($p < 0.05$) exhibited a stronger antioxidant activity compared to L-ascorbic acid (Figure 3) in a dose-dependent manner. The IC_{50} of the stem bark extract and L-Ascorbic acid were interpolated graphically as 28.0 mg/ml and 33.05 mg/ml, respectively

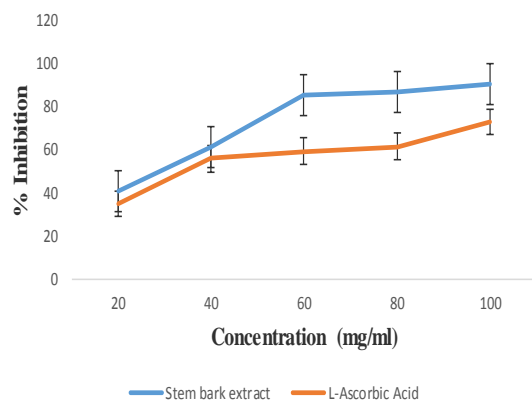


Figure 3: Ferric reducing antioxidant power of methanol stem bark extract of *F. sycomorus* plant and L-ascorbic acid. Each values is expressed as mean \pm SD, $n = 3$, ($p < 0.05$)

3.5. Hemolytic Inhibition Activity

Hemolysis induced by hydrogen peroxide in red blood cell (Figure 4) showed that the plant extract inhibited hemolysis at various concentrations of the extract used. The results showed decreases in hemolysis as extracts concentration increases in a dose-dependent manner.

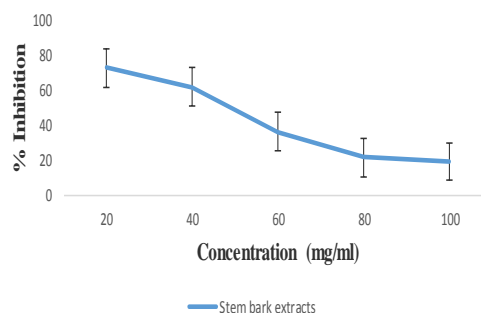


Figure 4: Hemolytic inhibition activity of *F. sycomorus* stem bark extracts. Values are expressed as mean \pm SD, $n = 3$, ($p < 0.05$)

3.6. Hemagglutination Inhibition Assay

The hemagglutination inhibition assay (Table 2) of different human blood groups was determined using various concentrations of *F. sycomorus* stem bark extract (20 - 100 mg/ml). The result showed low, moderate and strong activity of the extracts at 60 mg/ml, 80 mg/ml and 100 mg/ml for blood groups A, B, AB and O while no activity was observed at lower doses except for blood group B at 40 mg/ml.

Table 2. Hemagglutination inhibition assay of stem bark extract of *F. sycomorus* plant

Blood group	20 mg/ml	40 mg/ml	60 mg/ml	80 mg/ml	100 mg/ml	Buffer only
A+	-	-	-	+	+	-
B+	-	+	+	++	++	-
AB+	-	-	+	+	++	-
O+	-	-	+	++	+++	-

-: No activity; +: low activity; ++: moderate; +++: strong activity

3.7. Antibacterial Assay

Antibacterial activities of *F. sycomorus* stem bark extract were tested against five selected pathogenic organisms (Table 3). The result showed the inhibition percentage of the extract compared to the antibiotic drug Azithromycin at various concentrations of the extract. *E. coli* activity was significantly inhibited using 2 mg/ml extract (30.82 ± 8.73 %) compared to Azithromycin (14.69 ± 1.48 %). *S. aureus* and *S. typhi* activity were significantly inhibited by the extract at 2 mg/ml (30.23 ± 6.56 % and 30.49 ± 0.81 %), 4 mg/ml (40.31 ± 2.88 % and 36.99 ± 0.50 %) and 6 mg/ml (43.38 ± 0.94 % and 47.69 ± 1.02 %), respectively, while the antibiotic Azithromycin significantly inhibited the tested organisms at higher concentrations: 8 mg/ml (55.53 ± 1.23 % and 71.48 ± 1.11 %) and 10 mg/ml (63.37 ± 0.82 % and 75.00 ± 1.22 %). Azithromycin showed high inhibition against *B. cereus* compared to the stem bark extract while the pathogenic organism *P. aeruginosa* activity was significantly inhibited (41.82 ± 1.12 %, 49.02 ± 0.34 %, 56.03 ± 0.50 %, 69.90 ± 0.27 % and 73.26 ± 0.43 %) by the stem bark extract at the various concentrations used compared to the standard drug Azithromycin.

Table 3. Antibacterial activities (% Inhibition) of *F. sycomorus* stem bark extract

	Concentration	<i>E. coli</i>	<i>S. aureus</i>	<i>S. typhi</i>	<i>B. cereus</i>	<i>P. aeruginosa</i>
Stem bark extract	2 mg/ml	30.82 ± 8.73*	30.23 ± 6.56*	30.49 ± 0.81*	22.64 ± 1.63	41.82 ± 1.12*
	4 mg/ml	27.53 ± 2.18	40.31 ± 2.88*	36.99 ± 0.50*	32.79 ± 1.72	49.02 ± 0.34*
	6 mg/ml	30.85 ± 3.16	43.38 ± 0.94*	47.69 ± 1.02 *	44.57 ± 0.29	56.03 ± 0.50*
	8 mg/ml	43.63 ± 4.47	54.62 ± 2.76	53.47 ± 0.60	53.71 ± 0.29	69.90 ± 0.27*
	10 mg/ml	68.16 ± 6.98	62.25 ± 1.10	73.61 ± 1.06	57.33 ± 1.27	73.26 ± 0.43*
Azithromycin	2 mg/ml	14.69 ± 1.48	12.50 ± 0.41	18.40 ± 0.81	28.82 ± 0.80	14.12 ± 1.60
	4 mg/ml	37.50 ± 4.02	13.20 ± 3.03	21.77 ± 1.52	40.40 ± 0.40	18.68 ± 1.22
	6 mg/ml	42.05 ± 0.80	32.40 ± 2.24	23.57 ± 2.44	44.92 ± 1.20	41.95 ± 0.81
	8 mg/ml	59.66 ± 0.81	55.53 ± 1.23	71.48 ± 1.11	56.20 ± 2.52	42.24 ± 1.21
	10 mg/ml	64.21 ± 0.80	63.37 ± 0.82	75.00 ± 1.22	62.72 ± 0.79	55.46 ± 2.04

Values are Mean ± SD (N = 5); * Significant increased (p < 0.05) compared to Azithromycin

4. Discussion

Medicinal plants have a wide variety of phenolic compounds, such as flavonoids that act potentially as antioxidants, scavenging free radicals, reactive oxygen species and inhibit lipid peroxidation (Kumawat *et al.*, 2012). Antioxidants activities of stem bark extracts of *F. sycomorus* using DPPH radical scavenging activity, hydrogen peroxide scavenging activity and ferric reducing antioxidants power showed that the extracts significantly (p < 0.05) exhibited strong antioxidants activity compared to the standard (L-Ascorbic Acid) at the concentrations used. The results support the use of the plant therapeutically as well as economically as antioxidant additives or nutritional supplements and explored for novel antioxidants (van Wyk, 2008). The antiradical activity of the extracts could be related to the high content of tannins and flavonoids. The effects of the extracts could be due to the biological systems that are linked to their ability to transfer electrons to free radicals, chelate metals, activate antioxidant enzymes, reducing radicals of alpha-tocopherol or to inhibit oxidases (Bruneton, 2009). The results support studies on antioxidant activity of *Ficus pyriformis* extract and MeOH fraction possessing good scavenging activity compared to reference standards (ascorbic acid and quercetin) (Zedan *et al.*, 2015). The MeOH fraction showed a maximum activity in comparison with the other fractions. The antioxidants activity exhibited by the extract could be due to the presence of poly-phenolic compound such as flavonoids. The presence of ortho-dihydroxyl of the B-ring (3', 4'-di OH) of flavonoid molecule confers high stability to the flavonoid phenoxy radical, C2-C3 double bond in conjugation with 4-oxo group of the ring C participates in radical stabilization via electron delocalization over all three ring system. The presence of both 3- and 5-hydroxyl moiety of the rings C and A play an important role in radical scavenging activity of the flavonoids (Lv *et al.*, 2013).

Studies on phytochemical components confirmed the presence of tannins, saponins, flavonoids, terpenoids, phenols and steroids while glycoside and proteins were absent. The presence of these constituents may be attributed to the antioxidants activity of the extracts.

Medicinal plants contain different phytochemicals with biological activities having valuable therapeutic index. Therapeutic effects of this medicinal plant are attributed to the presence of their phytochemicals which are non-nutrient plant compounds. The major phytochemicals revealed are known to possess a wide range of activities, which may help in protection against chronic diseases. Saponins, flavonoids, tannins and alkaloids are known to possess hypoglycemic and anti-inflammatory activities, saponins also possess hypocholesterolemic and antidiabetic properties (Augusti *et al.*, 2008) while terpenoids decreases blood sugar levels in animal studies, steroids, triterpenoids and saponins showed analgesic properties and central nervous system activities (Argal *et al.*, 2006).

The antihemolytic inhibition assay of the extracts showed inhibitory effect on hydrogen peroxide induced hemolysis. The result showed a decrease in hemolysis as the concentration of the extract increases. The inhibitory effect may be attributed to their phenolic compounds which can donate electrons to H₂O₂, thus neutralizing it to water molecule (Alinezhad *et al.*, 2012). The antihemolytic inhibition of the extract was exhibited in a dose-dependent manner. Hemagglutination inhibition assay of the extracts exhibited a moderate (++) and a strong activity (+++) at a higher concentration (80 and 100 mg/ml) against the blood groups. The extracts may contain lectins and could be a useful source of important phytolectins. The hemagglutination activity is usually attributed to a group of proteins called lectins which are valuable agents for the separation and characterization of glycoconjugates and glycopeptides (Khan *et al.*, 2005).

The antibacterial activity (% inhibition) of the extract was tested against some organisms. The results revealed that the extracts significantly (p < 0.05) inhibited *S. aureus* and *S. typhi* at 2, 4, and 6 mg/ml, respectively. *P. aeruginosa* activity was significantly (p < 0.05) inhibited by the extract at 2, 4, 6, 8 and 10 mg/ml while the extract significantly (p < 0.05) inhibited *E. coli* at 2 mg/ml. The antibacterial activity exhibited could be due to the presence of phytochemicals (flavonoids, saponins, terpenoids, phenol and tannins) and the occurrence of phenolic compounds in the extract (Ramde-Tiendrebeogo *et al.*, 2012).

5. Conclusion

Studies on the phytochemical, antioxidant, antihemolytic, hemagglutination inhibition and antibacterial activities of *F. sycomorus* stem bark extract showed that the extract is a beneficial sources of phytochemicals. The results showed that the extract exhibited a strong antioxidant activity compared to L-ascorbic acid. Hemolytic inhibition assay and hemagglutination inhibition showed that the extract has a strong receptor binding affinity on erythrocytes. The plant extract exhibited a strong antibacterial activity against the human pathogenic organisms investigated. The study, therefore, supports the therapeutic uses of the plant in traditional medicine and suggest the need to isolate, identify and characterize the active principles responsible for its activity.

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