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Comparative biochemical evaluation of leaf extracts of *Ficus sycomorus* and *Piliostigma thonningii* plant

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Abstract

The study examined the phytochemical composition, DPPH radical scavenging activity, hydrogen peroxide scavenging radical activity, ferric reducing power, hemolytic inhibition, hemagglutination inhibition and antibacterial activity of methanol leaf extract of *Ficus sycomorus* and *P. thonningii* plant. The results showed the presence of tannins, saponins, flavonoids, glycoside and phenols while terpenoids, proteins and steroids were absent. The methanol leaf extract of the plants exhibited strong antioxidant activity measured using DPPH radical scavenging activity (Figure 1), hydrogen peroxide radical scavenging activity (Figure 2) and Ferric reducing power assay (Figure 3) at various concentrations of the extract used (20, 40, 60, 80 and 100 mg/ml). The hemolytic inhibition assay (Figure 4) and hemagglutination inhibition assay (Table 2 and 3) of the plants exhibited strong receptor binding affinity on human red blood cells. The methanol extract from the plants exhibited antibacterial activity (% inhibition) (Table 4) against five selected pathogenic organisms (*E. coli*, *S. aureus*, *S. typhi*, *B. cereus* and *P. aeruginosa*) at 2.0, 4.0, 6.0, 8.0 and 10 mg/ml concentrations of the extract. Our findings support the use of *F. sycomorus* and *P. thonningii* plant in the management and treatment of various ailments.

Keywords: *Ficus sycomorus*, *P. thonningii*, Antioxidant activity, Anti-hemolytic assay, Hemagglutination inhibition, Antibacterial activity

1. Introduction

Ficus sycomorus Linn belongs to the family *Moraceae*, comprising of about 40 genera and over 1,400 species of trees, shrubs, vine and herbs, often with milky latex juices. The plant is usually found near streams in many African countries. *F. sycomorus* tree grows up to 20 m with widely spreading branches and crown. *F. sycomorus* leaves are heart-shaped with a round apex, 14 cm long by 10 cm wide. The flowering and fruiting usually occurs from July to December and its foliage is widely consumed by sheep and cattle (Sarg *et al.*, 2011) [23]. *Ficus sycomorus* is used in Nigeria, Niger, Mali, South Africa, Guinea, Kenya, Tanzania, Somalia, Ethiopia and Ivory Coast as extract of fruits, leaf, root and stem barks to treat various ailments such as cough, diarrhea, skin infections, stomach disorders, liver disease, epilepsy, tuberculosis, lactation disorders, helminthiasis, infertility, sterility and diabetes mellitus (Igbokwe *et al.*, 2010 and Adoum *et al.*, 2012) [17, 2]. The plant has also been reported to be a potent antimicrobial agent against ciprofloxacin resistant *Salmonella typhi* (Adeshina *et al.*, 2010) [3]. In country like Palestine, milky sap from *F. sycomorus* stem bark is used for treating skin diseases while the decoction of the stem bark is used for problems of the gastrointestinal tract. It is further used as seasoning; the leaf are dried and added to cake as a condiment, eaten raw or cooked as soup. Dry branches of the species are collected and used as fuel (Auda, 2012) [8]. Nkafamiya *et al.*, (2010) [19] reported that the dry leaf of *F. sycomorus* leaf contained high amount of both protein and crude fibers while ash, lipid and carbohydrate contents were within the range expected for dry leafy vegetables. The study investigated the antioxidant and bioassay activities of methanol leaf extract of *F. sycomorus* plant.

Piliostigma thonningii (Schum) is a plant used for its medicinal purposes in many African countries. The parts of the plant have been used traditionally for the treatment of various diseases in humans and animals (Djuma, 2003) [15]. The roots and twigs are used in the treatment of wounds, dysentery, snake bites, respiratory ailments, hookworms and skin diseases. The leaves are used for the treatments of wounds, chronic ulcers, diarrhea, toothache and gingivitis, cough, and bronchitis. The leaf, stem bark or root extracts are taken as cough medicine, whereas the leaf extract as menorrhagia medicine. Alkaloids, flavonoids, saponins and tannins have already been isolated from the leaves of *P. thonningii*. Alfred, (2013) [5] reported that the leaves from the tree possess antibacterial, antimicrobial and antioxidant activities.

Ighodaro *et al.*, (2012) [18] reported that the dry leaf powder has been reported to contain alkaloids, saponins, flavonoids and tannins. Apart from carbohydrates, glycosides, flavonoids, tannins, saponins, balsams, volatile oil and terpenes have also been isolated from the leaves of *P. thonningii* (Egharevba *et al.*, 2010) [16].

The present study investigates the antioxidants, antihemolytic, hemagglutination and antibacterial activity of methanol leaf extracts from *F. sycomorus* and *P. thonningii* plants.

2. Materials and methods

2.1 Collection of plant material

Fresh leaf of *F. sycomorus* and *P. thonningii* plant was collected from around Sangere village, Girei Local Government Area, Adamawa State. The plant was taxonomically identified and authenticated in the Plant Science Department of Modibbo Adama University of Technology, Yola. The leaf 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 samples

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) [26].

2.4 Determination of DPPH (2, 2-diphenyl-2-picrylhydrazyl) radical scavenging activity

DPPH radical scavenging capacity of the plant extract was determined according to the method described by Sasidharan *et al.*, (2007) [25]. The free radical scavenging activity of the extract was measured by decrease in the absorbance of methanol solution of DPPH. The DPPH radical scavenging method was used for the determination of the antioxidant capacity of the extract. Different concentration of the plant extract each (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). Different concentration of L-Ascorbic acid (20, 40, 60, 80 and 100 mg/ml) was used as the standard antioxidant. After 30 min incubation at room temperature, the absorbance values was measured at 517 nm on a spectrophotometer (VIS 721, Pec Medical, USA) and converted into the percentage antioxidant activity using the following equation: 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

Hydrogen peroxide scavenging activity was determined using the method described by Repon *et al.*, (2013) [21]. Briefly, 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 absorption at 230 nm using a spectrophotometer. Each extract (20-100 mg/ml) in phosphate buffer was added to hydrogen peroxide and

absorbance at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. L-Ascorbic acid was used for comparison. The percentage of hydrogen peroxide scavenging was calculated using the following:

(%) of H₂O₂ scavenging activity

$$= \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Ascorbic acid was used as a positive control.

2.6 Ferric reducing antioxidant power (FRAP assay)

In ferric reducing antioxidant power assay, various concentration (20, 40, 60, 80 and 100 mg/ml) of the methanol extract each was 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 min followed by addition of 1 ml of 10% trichloroacetic acid. The mixture was then centrifuged for 10 min at room temperature. The supernatant obtained (1 ml) was added with 1 ml of deionised water and 200 µl of 0.1% FeCl₃. The blank was prepared in the same manner as the sample except that 1% potassium ferricyanide was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm. The reducing power was expressed as an increase in A_{700 nm} after blank subtracted (Bancrejee *et al.*, 2008) [11].

2.7 Anti-hemolytic assay

Inhibition of H₂O₂ induced red blood cell hemolysis of methanol extract of the plants was determined by *in vitro* method described by Tavazzi *et al.*, (2001) [27]. The erythrocytes from human blood was separated by centrifugation and washed with saline or isotonic sodium phosphate buffer (pH 7.4) until the supernatant is colourless. The erythrocytes were then diluted with saline or phosphate buffer to give a 4% suspension. Varying amounts of the plants extracts (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 concluded after these time intervals by centrifugation and the extent of hemolysis was determined by measurement of the absorbance at 540 nm corresponding to hemoglobin liberation. Anti-hemolytic activity was expressed as the inhibition percentage and was calculated using the following formula:

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

Hemagglutination activity of the methanol leaf extracts of *F. sycomorus* and *P. thonningii* plant was tested against human erythrocyte blood groups A⁺, B⁺, AB⁺ and O⁺ as described by Saha *et al.*, (2009) [24]. Stock solution of the test samples each 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. 1 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 negative activity, while a rough granular deposition at bottom showed positive activity. The intensity of hemagglutination was determined from the extent of deposition.

2.9 Antibacterial activity

The antibacterial activity of the methanol leaf extract of *F. sycomorus* and *P. thonningii* plant was determined as described by Akinyemi *et al.*, (2005) [6] using the modified broth dilution technique method. Five test tubes each were dispensed with 2 ml of sterile Nutrient broth followed by addition of 0.1 ml of standardized inoculums of test organisms (*E. coli*, *S. aureus*, *S. typhi*, *B. cereus* and *P. aeruginosa*) to each test tube. Various concentrations (2.0, 4.0, 6.0, 8.0 and 10.0 mg/ml) of the leaf extract each were added and the test tubes 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 activities of the *F. sycomorus* and *P. thonningii* extracts, antibiotic control and organism control was read using a colorimeter at 490 nm. Percentage growth inhibition was computed using the following given formula:

Percentage growth Inhibition (C)

$$= 100 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

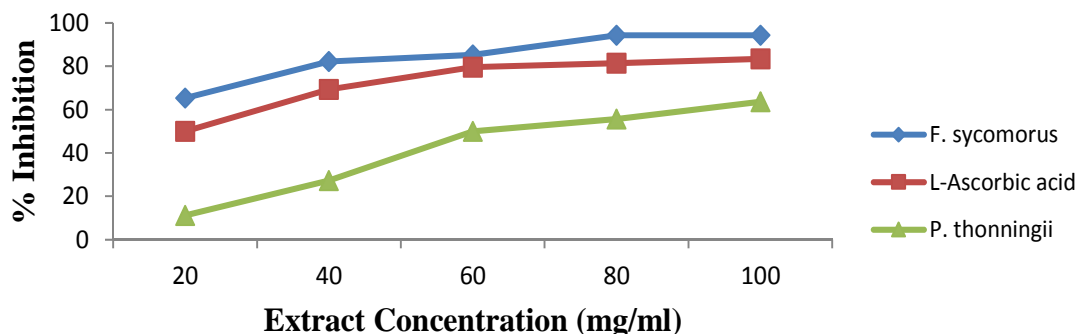


Fig 1: DPPH radical scavenging activity of Methanol Leaf Extract of *F. sycomorus*, *P. thonningii* plant and L-Ascorbic acid

3.3 Hydrogen peroxide radical scavenging activity

Hydrogen peroxide scavenging activity of the leaf extracts was measured in comparison with L-ascorbic acid (Figure 2). We discovered that both leaf extracts exhibited strong scavenging

3. Results

3.1 Phytochemical screening

Preliminary phytochemical screening of *F. sycomorus* leaf extract showed the presence of alkaloids, tannins, saponins, flavonoids, glycosides, proteins, phenols while terpenoids and steroids were absent. Phytochemical screening of *P. thonningii* leaf extract showed that alkaloids, tannins, saponins, flavonoids, glycosides, phenols while terpenoids, proteins and steroids were absent (Table 1).

Table 1: Phytochemical composition of methanol leaf extract of *F. sycomorus* and *P. thonningii* plant

Phytochemicals	<i>Ficus sycomorus</i> leaf extract	<i>P. thonningii</i> leaf extract
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
Terpenoids	-	-
Flavonoids	+	+
Glycosides	+	+
Proteins	+	-
Phenols	+	+
Steroids	-	-

+ = Present; - = Absent

3.2 Determination of DPPH radical scavenging activity

DPPH radical scavenging activity of the leaf extract of *F. sycomorus* and *P. thonningii* plant (Figure 1) showed that both leaf extracts exhibited radical scavenging activity at various concentration (20, 40, 60, 80 and 100 mg/ml). *F. sycomorus* leaf extract showed stronger antioxidant activity compared to L-Ascorbic acid and *P. thonningii* leaf extract

activity compared to L-ascorbic acid. However, *F. sycomorus* leaf extract showed stronger radical scavenging activity compared to *P. thonningii* leaf extract.

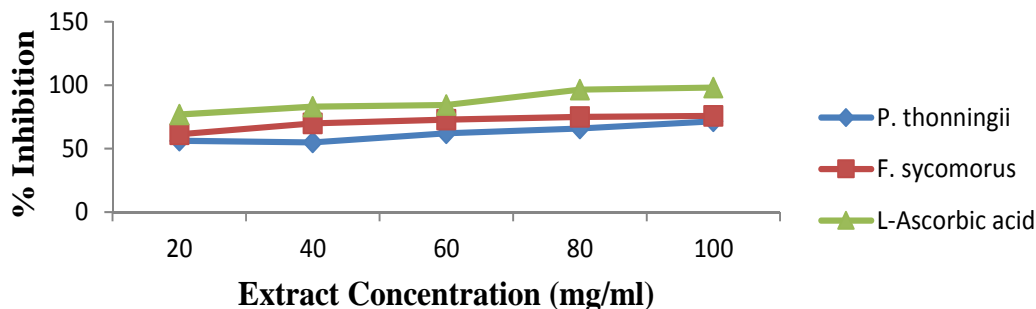


Fig 2: Hydrogen peroxide scavenging activity of methanol leaf extract of *F. sycomorus*, *P. thonningii* plant and L-Ascorbic acid

3.4 Ferric reducing antioxidant power

The ferric reducing assay of leaf extracts was studied and compared with L-ascorbic acid (Figure 3). The result showed that *P. thonningii* methanol leaf extract showed stronger

radical scavenging activity compared to *F. sycomorus* leaf extract and L-ascorbic acid. Both plants extract exhibited significant radical scavenging activity compared to L-Ascorbic acid.

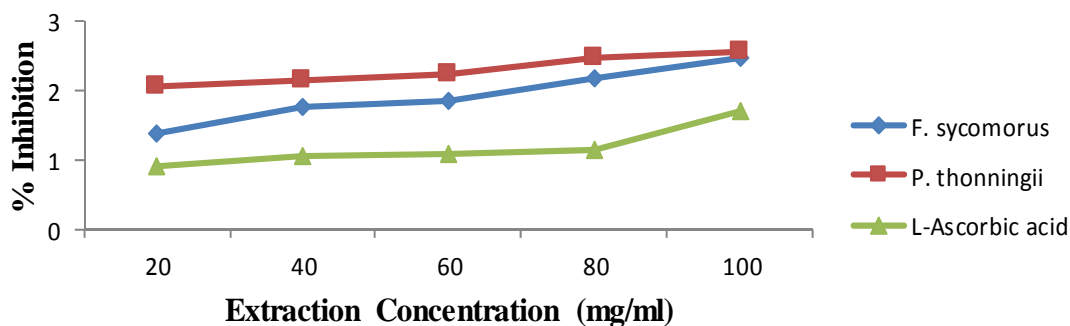


Fig 3: Ferric reducing antioxidant power of methanol leaf extract of *F. sycomorus*, *P. thonningii* plant and L-ascorbic acid

3.5 Hemolytic inhibition activity

Induced hemolysis by hydrogen peroxide in red blood cell was shown (figure 4). The methanol extracts of *F. sycomorus* and

P. thonningii plant was shown to be inhibited at various concentrations used. *F. sycomorus* leaf extracts showed stronger inhibition compared to *P. thonningii* leaf extract.

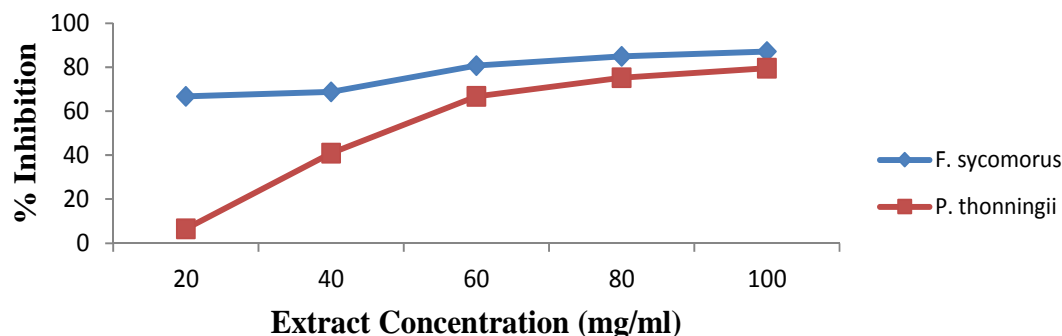


Fig 4: Hemolytic inhibition activity of *F. sycomorus* and *P. thonningii* leaf extract

Table 4: Antibacterial activity of leaf extracts of *Ficus sycomorus* and *P. thonningii* plants (% Inhibition)

	Extract (mg/ml)	<i>E. coli</i>	<i>S. aureus</i>	<i>S. typhi</i>	<i>B. cereus</i>	<i>P. aeruginosa</i>
<i>F. sycomorus</i> extract	2.0	30.04 ± 3.68	9.15 ± 1.76	14.47 ± 4.09	9.65 ± 0.67	7.55 ± 3.97
	4.0	34.37 ± 5.17	29.73 ± 9.23	32.98 ± 8.66	16.36 ± 4.75	45.06 ± 3.29
	6.0	38.83 ± 6.37	44.15 ± 11.78	38.91 ± 7.14	37.31 ± 3.90	45.80 ± 4.00
	8.0	46.12 ± 1.92	45.51 ± 1.34	47.94 ± 8.28	38.11 ± 1.39	52.86 ± 9.45
	10.0	50.24 ± 5.81	51.97 ± 1.99	52.32 ± 3.82	47.31 ± 9.07	54.66 ± 6.63
<i>P. thonningii</i> extract	2.0	8.04 ± 0.88	10.47 ± 1.54	16.73 ± 0.54	9.32 ± 0.79	11.86 ± 0.78
	4.0	12.44 ± 1.15	12.45 ± 1.13	18.11 ± 0.35	11.42 ± 1.39	17.12 ± 0.74
	6.0	13.04 ± 0.71	17.12 ± 0.81	24.85 ± 0.88	12.94 ± 1.06	20.03 ± 0.71
	8.0	18.34 ± 1.23	18.21 ± 0.33	28.52 ± 0.49	13.70 ± 1.37	28.79 ± 0.63
	10.0	32.19 ± 3.75	34.49 ± 1.06	34.49 ± 1.06	25.71 ± 0.73	32.29 ± 0.61
Azithromycin	2.0	14.69 ± 1.48	12.50 ± 0.41	18.40 ± 0.81	28.82 ± 0.80	14.12 ± 1.60
	4.0	37.50 ± 4.02	13.20 ± 3.03	21.77 ± 1.52	40.40 ± 0.40	18.68 ± 1.22
	6.0	42.05 ± 0.80	32.40 ± 2.24	23.57 ± 2.44	44.92 ± 1.20	41.95 ± 0.81
	8.0	59.66 ± 0.81	55.53 ± 1.23	71.48 ± 1.11	56.20 ± 2.52	42.24 ± 1.21
	10.0	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)

3.6 Hemagglutination inhibition assay

The hemagglutination inhibition activity of different human blood groups was determined at various concentrations of the plant extract (20 - 100 mg/ml). The hemagglutination

inhibition activity of *F. sycomorus* leaf extract as tested against four different human blood groups (Table 2).

Table 2: Hemagglutination inhibition assay of leaf 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

Table 3: Hemagglutination inhibition assay of leaf extract of *P. thonningii* 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

3.7 Antibacterial assay

The antibacterial activity of *F. sycomorus* and *P. thonningii* leaf extract was tested against five selected pathogenic organisms and the results presented in Table 3. The antibacterial activity shows the percentage inhibition of the leaf extracts at various concentrations used compared to standard drug Azithromycin. The extract of *F. sycomorus* leaf at 2 mg/ml significantly inhibited *E. coli* while *S. aureus* and *S. typhi* were significantly inhibited by the extract at 4 mg/ml and 6mg/ml respectively compared to Azithromycin. The result showed that *P. aeruginosa* was significantly inhibited by the extract at 4 mg/ml, 6mg/ml and 8mg/ml compared to Azithromycin. Azithromycin at concentrations (2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml and 10 mg/ml) significantly inhibited *B. cereus* compared to the leaf extract of *F. sycomorus* plant. Antibacterial activity of methanol leaf extract of *P. thonningii* plant showed percentage inhibition of extract at various concentrations used. *P. thonningii* plant extract inhibited *S. typhi* at 6 mg/ml compared to the Azithromycin. Azithromycin showed higher zone of inhibition compared to the methanol extract of *P. thonningii* plant at various concentrations used.

4. Discussion

Medicinal plants and herbs are recognized for their wide variety of phytochemicals and phenolic compounds such as flavonoids that act as an antioxidant to scavenge free radicals and inhibit lipid peroxidation. The benefits of medicinal plants therapeutically have been attributed to its antioxidant properties and have been utilized economically as antioxidant additives or nutritional supplements (Thagriki *et al.*, 2015) [28]. Our report on the radical scavenging activity of *F. sycomorus* and *P. thonningii* leaf extracts using DPPH radical scavenging activity, Hydrogen peroxide radical scavenging activity, Ferric reducing antioxidant power and anti-hemolytic activity showed that the plant extract possess antioxidants which may be attributed to the presence of their phytochemicals. Major phytochemicals present in the methanol leaf extract of *F. sycomorus* and *P. thonningii* leaf extracts are known to possess a wide range of activities, which may help in the protection against chronic diseases. Tijjani *et al.*, (2012) [30] reported the presence of alkaloids, tannins, saponins and glycoside in the leaf extract *P. thonningii* plant while Tibiri *et al.*, (2012) [29] reported the presence of alkaloids, flavonoids, tannins, saponins, and sterols in the leaf extract of *F. sycomorus* plant.

The studies on the antioxidant activity parameters, anti-hemolytic assay and hemagglutination inhibition assay of *F. sycomorus* and *P. thonningii* leaf extracts showed that the extract has strong antioxidant activity, hemolytic inhibition and hemagglutination inhibition. Our studies support previous reports by (Al-Fatimi *et al.*, 2007; Abdel-Hameed, 2009) [1, 4] on the antioxidant activity of some species of *Ficus* plant including the *F. sycomorus*. The antiradical activity of *F. sycomorus* and *P. thonningii* leaf extracts could be due to the presence of phenolic compounds (phenols and flavonoids). Flavonoids have been reported as important antioxidant and antiradical activities. The radical scavenging effect of the plant 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) [13]. Carriere *et al.*, (2004) reported the effects of hypoxia are partially mitigated in response to treatment with antioxidants which their primary sources are majorly plant sources. Bouchet *et al.*, (1998) [12] reported that hydrolysable or condensed tannins have antiradical and antioxidant properties expressed by their inhibiting effect on lipid peroxidation (induced by Fe²⁺) and radical-scavenging ability on DPPH radical. *F. sycomorus* and *P. thonningii* leaf extracts have binding affinity to the receptors of erythrocytes and prevent agglutination.

The results showed that *F. sycomorus* and *P. thonningii* leaf extract has antibacterial activity. Barku *et al.*, (2013) [10] reported the antibacterial activity of the plants extract may be due to the presence of metabolic toxins and broad spectrum antimicrobial compounds that may act against bacteria. The antibacterial activity of the plants extract may be due to the strong extraction capacity of methanol which produced greater numbers of active constituents responsible for antibacterial activity.

5. Conclusion

We concluded that *F. sycomorus* and *P. thonningii* leaf extract contained various phytochemicals which confirmed the use of these plants in traditional medicine and for therapeutic purposes. The presence of these phytochemicals in the extracts may be responsible for their radical scavenging activity leading to the inhibitory erythrocytes hemolysis, hemagglutination and antibacterial activity. The antibacterial activity of the leaf extracts against the test organisms also supports its exploitation in traditional system of medicine practice.

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