Qualitative and Quantitative Analyses of Phytochemicals and Antioxidant Activity of Ficus sagittifolia (Warburg Ex Mildbread and Burret)

Taiwo O. Margaret, Olaoluwa O. Olaoluwa

Abstract—Moraceae family has immense phytochemical constituents and significant pharmacological properties, hence have great medicinal values. The aim of this study was to screen and quantify phytochemicals as well as the antioxidant activities of the leaf and stem bark extracts and fractions (crude ethanol extracts, n-hexane, ethyl acetate and aqueous ethanol fractions) of Ficus sagittifolia. Leaf and stem bark of F. sagittifolia were extracted by maceration method using ethanol to give ethanol crude extract. The ethanol crude extract was partitioned by n-hexane and ethyl-acetate to give their respective fractions. All the extracts were screened for their phytochemicals using standard methods. The total phenolic, flavonoid, tannin, saponin contents and antioxidant activity were determined by spectrophotometric method while the alkaloid content was evaluated by titrimetric method. The amount of total phenolic in extracts and fractions were estimated in comparison to gallic acid, whereas total flavonoids, tannins and saponins were estimated corresponding to quercetin, tannic acid and saponin respectively. 2, 2-diphenylpicryl hydrazyl radical (DPPH)* and phosphomolybdate methods were used to evaluate the antioxidant activities of leaf and stem bark of F. sagittifolia. Phytochemical screening revealed the presence of flavonoids, saponins, terpenoids/steroids, alkaloids for both extracts of leaf and stem bark of F. sagittifolia. The phenolic content of F. sagittifolia was most abundant in leaf ethanol crude extract as 3.53 ± 0.03 mg/g equivalent of gallic acid. Total flavonoids and tannins content were highest in stem bark aqueous ethanol fraction of F. sagittifolia estimated as 3.41 ± 0.08 mg/g equivalent of quercetin and 1.52 ± 0.05 mg/g equivalent of tannic acid respectively. The hexane leaf fraction of F. sagittifolia had the utmost saponin and alkaloid content as 5.10 ± 0.48 mg/g equivalent of saponins and 0.171 ± 0.39 g of alkaloids. Leaf aqueous ethanol fraction of F. sagittifolia showed high antioxidant activity (IC_{50} value of 63.092 µg/mL) and stem ethanol crude extract (227.43 ± 0.78 mg/g equivalent of ascorbic acid) for DPPH and phosphomolybdate method respectively and the least active was found to be the stem hexane fraction using both methods (313.32 µg/mL; 16.21 ± 1.30 mg/g equivalent of ascorbic acid). The presence of these phytochemicals in the leaf and stem bark of F. sagittifolia are responsible for their therapeutic importance as well as the ability to scavenge free radicals in living systems.

Keywords—Antioxidant activity, Ficus sagittifolia, Moraceae, phytochemicals.

I. INTRODUCTION

Phytochemicals formed by living systems, notably from plant origin have shown great potential in treating human diseases such as cancer, coronary heart diseases, diabetes and infectious diseases. They are responsible for the biological activity in the plant host and play a role in plant growth or defense against competitors, predators or pathogens [1], [2]. Reactive oxygen species (ROS) generate free radicals which cause damage to other molecules by abstracting electrons from them in order to achieve stability. All aerobic organisms produce ROS which are very reactive with most biomolecules (proteins, lipids, nucleic acids). ROS include superoxide radical, hydroxyl radical, nitric oxide radical, lipid peroxyl radical and non-free radical species such as hydrogen peroxide, singlet oxygen, ozone, lipid peroxide are different forms of activated oxygen [3]-[5]. Most plants contain free radical scavenging molecules which are responsible for their immense antioxidant properties [6], [7]. Scientific findings have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, antitumor, antiulcer, anti-mutagenic, anti-carcinogenic, antibacterial and antiviral activities [8], [9]. The intake of natural antioxidants has been linked with reduced risks of cancer, cardiovascular disease, diabetes, ulcer and other diseases associated with ageing [10], [11]. In recent years, the use of natural phytochemicals has been a trend worldwide since natural antioxidants are presumed to be safe as they occur in plants. Scientific reports suggested that compounds, mainly from natural sources, are capable of providing defense against free radicals [11]. This has attracted a great deal of research interest in natural antioxidants.

One of the largest genus of Moraceae (mulberry) family is Ficus, consisting of more than 800 species. It is found in lowland rainforest of tropical region [12]. Different parts of this genus including leaves, bark, root, fruits and latex are frequently used for treatments of ailments related to digestive, endocrine, reproductive and respiratory systems. Some common species of Ficus include: F. retusa, F. sycomorus, F. benghalensis, F. religiosa, F. carica, F.exasperata, F. auriculata, F. conraui, F. polifo, F. capensis and F. asperifolia, F. elastic. These species have great medicinal values as they have been reported to have enormous phytochemicals including tannins, flavonols and flavonoids, terpenoids, phenols, coumarins, glycosides, esters, carbohydrates, serine protease and also possess significant pharmacological properties such as antimicrobial, anti-

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diabetic, antiulcer, antioxidant and anticancer [13]. Most of
the studies of the Ficus species revealed the presence of
phenolic compounds as major components from different parts
(leaves, stem wood, branches, stem bark, roots, root bark,
fruits, and seeds) [14].

Ficus sagittifolia (Warb. ex Mildbr. & Burret) is a member
of the genus, Ficus and from the family, Moraceae. It is a
shrub, epiphytic, often on oil palms, becoming a tree to 10 m
high; in the closed-forest in Casamance Senegal, Nigeria and
Cameroon. The bark and leaf are used as a cure for pulmonary
and stomach troubles respectively. In Sierra Leone, the bark is
edible and is taken as colic [15]. Interestingly, sparse
information has been reported about this Ficus specie with
medicinal virtues. In continuation of our effort to provide
scientific rationale for the use of medicinal plants, hence, this
paper reports the qualitative and quantitative analyses of
phytochemicals as well as antioxidant activity of F. sagittifolia.

II. MATERIALS AND METHODS

A. Collection of Plants

Fresh leaves and stem bark were harvested in April, 2018
from Ikere, Osun state, Nigeria. Identification of the plant
parts was carried out in the herbarium section of Forest Research
Institute of Nigerian (FRIN), Ibadan. Plant specimen, with
reference number FHI 111988, was deposited in the herbarium
by Mr S. A. Odewo

B. Processing and Preparation of Plant Samples

The leaves and stem bark were air dried at room
temperature. The dried plant materials were then pulverized
using a laboratory miller. The two plant parts were separately
subjected to organic extractions using ethanol by cold
extraction method.

C. Extraction from Stem Bark

About 1.0 kg of the stem bark of Ficus sagittifolia was
extracted by cold extraction method at room temperature for
72 h using ethanol. The extract was decanted, fresh solvent
was added and the maceration process repeated until the
material was exhausted. The filtrate was concentrated using a
rotary evaporator at 38-40 °C. The concentrate was then
evaporated to dryness using a desiccator. The weight of the
crude extract was 51 g and a yield of 5.1% was obtained.

D. Extraction from Leaves

A quantity of 1.8 kg of the coarsely powdered leaves of
Ficus sagittifolia was cold macerated using ethanol (10L) for
72 hrs in a sealed aspirator bottle. The extract was decanted
and concentrated under pressure using a rotary evaporator at
38-40 °C. The concentrate was further evaporated to dryness
using a desiccator. The weight of crude extract obtained was
69 g giving a yield of 3.83%.

E. Ethanolic Crude Sample Partitioning

Kupchan liquid-liquid partitioning was employed using a
separating funnel. This method separates compounds based on
their relative solubility in two different immiscible solvents
[16]. Leaves and stem bark crude samples were partitioned
separately with n-hexane and ethyl acetate to obtain n-hexane
fraction, ethyl acetate fraction and aqueous ethanolic fraction.

F. Quantitative Phytochemical Analysis

Small quantity of the ethanol crude extracts and fractions
(hexane, ethyl acetate and aqueous ethanol fraction) from F.
sagittifolia leaves and stem bark were subjected to the
preliminary phytochemical analysis following standard
methods by [17]. The ethanol crude extracts and fractions
were screened to detect the presence of various active
principles like terpenoids, flavonoids, alkaloids, steroids,
cardiac glycoside, tannins, saponins, anthraquinones,
phylobatannins and carbohydrates (reducing sugar) [17].

G. Quantitative Phytochemical Analysis

Total Phenolic Content Determination: Total phenolic
content (TPC) was determined by the spectrophotometric
method using Folin-Ciocalteau assay [18]-[20]. A volume of 1
mL of leaves and stem bark F. sagittifolia crude extracts and
their fractions were mixed separately with 1 mL of Folin-
Ciocalteau’s phenol reagent. Sodium carbonate solution of 7% and
13 mL of distilled water were added after 5 min and mixed
thoroughly. The mixture was incubated for 90 min at 25 °C
and absorbance values were taken at 750 nm. These were
carried out in triplicates. TPC was determined from a standard
curve of Gallic acid solution (y = 0.213x + 0.6469; R 2 =
0.6469) and expressed as milligrams of Gallic acid equivalents
(GAE)/g of dried sample.

Total Flavonoid Content Determination: Total flavonoid
content was determined following method of Park et al. [21].
A volume of 0.3 mL each of leaves and stem bark F.
sagittifolia crude extracts and their fractions, 3.4 mL of 30%
methanol, 0.15% of NaNO2 (0.5M) and 0.5 mL of AlCl3.6H2O
(0.3M) were mixed. After 5 min, 1 mL of NaOH (1M) was
added. The mixture was kept at room temperature for 30 min;
the absorbance of the reaction mixture was measured at 506
nm using UV/visible spectrophotometer. This was done in
triplicate and calculated using standard graph of quercetin (y =
0.296x + 0.053; R2 = 0.6469) and the results were expressed as
quercetin equivalent (mg/g).

Total Saponin Determination: About 0.5 g of leaves and
stem bark of F. sagittifolia crude extracts and their fractions
were separately weighed followed by the addition of distilled
water. Each mixture was shaken and allowed to stand for an
hour. The formation of a stable foaming froth was observed.
About 1 mL of the mixture was pipetted into another test tube
with about 5 mL of distilled water added, followed by the
addition of olive oil and was shaken to obtain a cloudy
appearance. The absorbance was measured at 620 nm using
spectrophotometer. This was done in triplicate and calculated
using standard graph of saponin (y = 0.219x + 0.063; R2 =
0.7460) and the results were expressed as saponin equivalent
(mg/g).

Total Tannin Determination: Distilled water was added to a
small quantity of F. sagittifolia leaves and stem bark crude
extracts and their fractions separately and filtered, 5 mL of the filtered samples was measured followed by the addition of 2 mL of 0.1 M FeCl₃ in 0.1M HCl and 0.008M K₂Fe(CN)₆·3H₂O. Absorbances of the samples were measured using a spectrophotometer at 395 nm. This was done in triplicate. The results of tannins were expressed in terms of tannic acid in mg/g using standard graph of tannic acid (y = 0.917x + 0.398; R² = 0.6195) [22], [23].

Total Alkaloid Determination: Alkaloid content of *F. sagittifolia* leaves and stem bark crude exacts and their fractions were determined by titrimetric method. Obtained supernatant of the samples (10 mL of each) were taken into 10 mL of 0.1 N HCl in a flask and shaken thoroughly for 2-3 min. The lower layer contains alkaloids neutralized with 0.1 N HCl. The HCl portion (10 mL) was collected in a beaker and 2-3 drops of methyl red were added giving a slightly reddish colour. This was then titrated against 0.1N NaOH till colour changes from red to pale yellow. This was done in triplicate. The total amount of alkaloids was calculated by considering the following equivalent:

\[
1 \text{ mL} 0.1 \text{ N HCl} = 0.0612 \text{ g of alkaloid} \quad (1)
\]

\[H\] Antioxidant Activity

**DPPH Radical Scavenging Activity**: The DPPH radical scavenging assay was performed by the method of Koleva et al. [24]. The scavenging ability of the extracts and fractions were observed by disappearance of the purple color from the DPPH methanolic solution to pale yellow resulting to a decrease in absorbance. Extracts and fractions were weighed (10 mg) and then dissolved with methanol up to 10 mL to obtain a concentration of 1 mg/mL. Dilution was performed by adding distilled water so that the samples with concentrations of 100, 200, 300, 400, 500 μg/mL were obtained. For the determination of antioxidant activity, each concentrate (0.2 mL) was pipetted with a micro pipette and 3.8 mL of 50 μM DPPH methanolic solution was added. The decrease in absorbance of the extracts and fractions due to quenching of DPPH free radical was measured at 517 nm. The IC₅₀ values were determined as the concentration of the extracts and fractions that gave 50% reduction in the absorbance from control blank. Ascorbic acid was used as a reference standard. The percentage inhibition was calculated as:

\[
(\% \text{ Inhibition}) = \frac{[\text{control} - \text{test}]}{\text{control}} \times 100
\]

**Total Antioxidant Capacity** (Phosphomolybdate Assay): The total antioxidant capacity was determined by phosphomolybdate method using ascorbic acid as standard [25]. A mixture of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate (reagent solution) were prepared and 1 mL was added to each sample. Incubation was done for 90 min at 95 °C. The samples were allowed to cool to room temperature and the absorbance of each sample mixtures was measured at 765 nm. A blank which contained 1 mL of reagent solution was also incubated under same condition. The results of total antioxidant capacity were expressed in terms of ascorbic acid in mg/g using standard graph of ascorbic acid.

I. Statistical Analysis

All the experiments were done in triplicates. The experimental results were expressed as mean ± SEM (standard error of mean) of triplets using Excel 2013 (Microsoft Corporation, Redmond, USA).

III. RESULTS AND DISCUSSION

A. Qualitative Phytochemical Analysis

Preliminary phytochemical screening of *F. sagittifolia* ethanolic leaf (FSL) and stem bark (FSS) crude extracts as well as their fractions – hexane (Hex), ethyl acetate (EtOAc) and aqueous ethanol fractions (Aq EtOH) revealed the presence of phenolics, flavonoids, steroids, tannins, saponins, alkaloids, and terpenoids (Table I). These phytochemicals have been reported to exhibit various therapeutic activities in medicine. Terpenoids promotes glutathione-S-transferase and cancer cell apoptosis; hence, terpenoids have been used for anti-cancer properties. Flavonoids are well known for their anti-viral, anti-inflammatory, antioxidant activities and also used in the treatment of hypertension, diabetes, ulcer, rheumatic fever [26]. Polyphenols are active in curing kidney and stomach problems and have been found to be helpful in protection and prevention against many diseases. [27]. Steroids are known important for their cardio-tonic activities and also used in nutrition, herbal medicine and cosmetics [28]. Alkaloids and their derivatives are very important and are used in analgesic, antispasmodic and bactericidal activities. Saponins have properties of precipitating and coagulating red blood cells and they also have cholesterol binding properties, formation of foams in aqueous solutions and hemolytic activity. Tannins have showed remarkable result in the treatment of inflamed tissues, diarrhea and dysentery; also as anticancer agents [27], [29].

B. Quantitative Phytochemical Analysis

TPC of ethanol crude extract (crude), hexane (Hex), ethyl acetate (EtOAc) and aqueous ethanol (Aq EtOH) fractions from *F. sagittifolia* leaf (FSL) and FSS were determined in terms of mg of Gallic Acid Equivalent (mg GAE/g) and the results were shown in Table II. The ethanolic crude extract of *F. sagittifolia* stem bark (FSS crude) had the highest TPC, next to the aqueous ethanol fraction of *F. sagittifolia* stem bark (FSS Aq EtOH) with the hexane fraction from *F. sagittifolia* leaf (FSL) showing the lowest TPC. The TPC in the order of decreasing content is as follows: FSS crude > FSS aq EtOH > FSS EtOAc > FSL Crude > FSL aq EtOH > FSL EtOAc > FSS Hex > FSL Hex. The total flavonoid content (TFC) of the extracts and fractions of FSL and stem bark were determined in terms of Quercetin Equivalent g as seen in Table II, the aqueous ethanol fraction from the stem bark of *F. sagittifolia* had the highest flavonoid content followed by ethanol crude extract as shown in decreasing order: FSS aq EtOH > FSS crude > FSL aq EtOH > FSL...
EtOAc > FSS EtOAc > FSL Crude > FSL Hex > FSS Hex. Phenolics (including many flavonoids) contain polar phenolic hydroxyl groups which are readily extracted by polar solvents such as water and ethanol. In these solvents, phenolic compounds lose a proton to produce a phenolate ion which reduces Folin-Ciocalteu reagent. The hexane leaf fraction of *F. sagittifolia* had the utmost saponins and alkaloids content as 5.10 ± 0.48 mg/g equivalent of saponin and 0.171 ± 0.39 g of alkaloids. The tannin content was prevalent in the aqueous ethanol fraction from the stem bark of *F. sagittifolia* estimated as 1.52 ± 0.05 mg/g equivalent of tannic acid. The stem bark of *F. sagittifolia* revealed high content of phenolics, tannins, flavonoids as compared to the leaves.

### TABLE I

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>FSL HEX</th>
<th>FSS HEX</th>
<th>FSL EtOAc</th>
<th>FSS EtOAc</th>
<th>FSL crude</th>
<th>FSS crude</th>
<th>FSL Aq ETOH</th>
<th>FSS Aq ETOH</th>
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<tr>
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<tr>
<td>Tannins</td>
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<tr>
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<td>Absent</td>
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<tr>
<td>Phlobatannins</td>
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<tr>
<td>Carbohydrate (Reducing sugar)</td>
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<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
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</table>

### TABLE II

<table>
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<tr>
<th>Compounds</th>
<th>Total phenolics (GAE/g)</th>
<th>Total flavonoids (QUE/g)</th>
<th>Total Saponins (SA/g)</th>
<th>Total Tannins (TA/g)</th>
<th>Total Alkaloids (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSL Hex</td>
<td>0.32±0.02</td>
<td>0.80±0.18</td>
<td>5.10±0.48</td>
<td>0.99±0.32</td>
<td>0.171±0.78</td>
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<tr>
<td>FSS Hex</td>
<td>0.47±0.05</td>
<td>0.37±0.08</td>
<td>3.95±0.07</td>
<td>1.31±0.02</td>
<td>0.058±0.14</td>
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<tr>
<td>FSL EtOAc</td>
<td>2.14±0.03</td>
<td>1.48±0.07</td>
<td>2.73±0.11</td>
<td>1.33±0.05</td>
<td>0.158±3.18</td>
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<tr>
<td>FSS EtOAc</td>
<td>2.12±0.11</td>
<td>1.20±0.01</td>
<td>2.59±0.46</td>
<td>0.99±0.31</td>
<td>0.168±1.27</td>
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<tr>
<td>FSL Crude</td>
<td>2.21±0.02</td>
<td>0.68±0.02</td>
<td>3.43±0.45</td>
<td>1.34±0.01</td>
<td>0.101±3.18</td>
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<td>FSS Crude</td>
<td>3.53±0.03</td>
<td>1.84±0.04</td>
<td>2.81±0.48</td>
<td>1.26±0.07</td>
<td>0.050±0.71</td>
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<td>FSL Aq ETOH</td>
<td>2.14±0.11</td>
<td>1.70±0.04</td>
<td>3.13±0.03</td>
<td>1.43±0.04</td>
<td>0.073±0.57</td>
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<tr>
<td>FSS Aq ETOH</td>
<td>3.25±0.03</td>
<td>3.41±0.08</td>
<td>2.42±0.23</td>
<td>1.52±0.05</td>
<td>0.070±0.49</td>
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</table>

C. Antioxidant Activity

DPPH Radical Scavenging Activity

The DPPH radical is widely used as the model system to investigate the scavenging activities of several natural compounds such as phenolic or crude extracts of plants. At ambient temperature, DPPH is a relatively stable free radical which becomes a stable diamagnetic molecule when it abstracts an electron or hydrogen radical [30]. DPPH radical is scavenged by antioxidants present in the FSL and stem bark through the donation of proton forming the reduced DPPH. IC₅₀ values were obtained based on linear regression equation (Table III; Fig. 1). The aqueous ethanol fraction of *F. sagittifolia* leaf (FSL Aq ETOH) had the lowest IC₅₀ DPPH scavenging activity (63.092 µg/mL) depicting to have the highest antioxidant activity and hexane fraction of *F. sagittifolia* stem (FSS Hex) was found to be the least in activity. The aqueous ethanol fraction showed prominent antioxidant activity than ethanol crude extract and ethyl acetate fraction which in turn was more active than the hexane fraction for both the leaf and stem bark of *F. sagittifolia*, the presence of phenolic compounds (containing phenolic hydroxyl) in these aqueous ethanol fractions could be attributed to the observed high antiradical properties of these fractions. The order of decreased activity is: FSL Aq ETOH > FSS Aq EtOH > FSS EtOAc > FSS Crude > FSL EtOAc > FSL Crude > FSL Hex > FSS Hex with their IC₅₀ values, 63.092, 65.025, 68.763, 70.561, 79.835, 84.732, 243.022, 313.323 µg/mL respectively.

### TABLE III

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>IC₅₀ (µg/mL)</th>
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<tbody>
<tr>
<td>FSL Hex</td>
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<tr>
<td>FSS Hex</td>
<td>313.32</td>
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<tr>
<td>FSL EtOAc</td>
<td>79.837</td>
</tr>
<tr>
<td>FSS EtOAc</td>
<td>68.763</td>
</tr>
<tr>
<td>FSL Crude</td>
<td>84.732</td>
</tr>
<tr>
<td>FSS Crude</td>
<td>70.561</td>
</tr>
<tr>
<td>FSL Aq EtOH</td>
<td>63.092</td>
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<tr>
<td>FSS Aq EtOH</td>
<td>65.025</td>
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<tr>
<td>Vitamin C</td>
<td>21.239</td>
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</table>

Phosphomolybdate Assay (Total Antioxidant Capacity)

The phosphomolybdate assay is a quantitative method used to determine the total antioxidant capacity (TAC) of plant extract/fraction usually expressed as ascorbic acid equivalents. The principle is based on the reduction of Mo(VI) to Mo(V)
by the plant extract/fraction at 765 nm. The antioxidant capacity of the ethanol crude extracts and fractions of FSL and stem bark were found to decrease in this order: FSS crude > FSS Aq EtOH > FSS EtOAc > FSL crude > FSL Aq EtOH > FSL EtOAc > FSL Hex > FSS Hex fraction. Ethanol crude extract of *F. sagittifolia* stem exhibited the highest antioxidant capacity for phosphomolybdate reduction (227.424 ± 0.78) with the hexane fraction been the least in activity (16.212 ± 1.30), Table IV. Recent studies have shown that many flavonoids and related polyphenols contributed significantly to the phosphomolybdate scavenging activity of medicinal plants [22], [31].

**TABLE IV**

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>AAE (mg/g)</th>
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<td>FSL HEX</td>
<td>18.03±0.81</td>
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<tr>
<td>FSS HEX</td>
<td>16.21±1.30</td>
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<tr>
<td>FSL EtOAc</td>
<td>103.78±1.53</td>
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<tr>
<td>FSS EtOAc</td>
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<tr>
<td>FSL crude</td>
<td>134.92±8.62</td>
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<tr>
<td>FSS crude</td>
<td>227.42±0.78</td>
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<tr>
<td>FSL Aq EtOH</td>
<td>134.24±2.47</td>
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<tr>
<td>FSS Aq EtOH</td>
<td>193.10±0.77</td>
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</tbody>
</table>

Fig. 1 IC₅₀ values obtained based on linear regression equation for *F. sagittifolia* leaf and stem bark extracts and fractions: FSL- *Ficus sagittifolia* Leaf; FSS- *Ficus sagittifolia* Stem; HEX- hexane; ETOH- ethanol; Aq ETOH- aqueous ethanol

**IV. CONCLUSION**

The present investigation revealed the presence of various phytochemicals such as terpenoids, flavonoids, phenols, alkaloids, saponins and steroids in the leaf and stem bark of *F. sagittifolia*. The aqueous ethanol fraction of leaf and stem bark of *F. sagittifolia* showed good antioxidant activity among others using DPPH radical scavenging and phosphomolybdate method while the least activity were observed in the hexane fractions. Hence, aqueous ethanol fractions of leaf and stem bark of *F. sagittifolia* are good sources of antioxidants.

**ACKNOWLEDGMENT**

Authors acknowledge the use of J-laboratory facilities, Department of Chemistry, University of Ibadan, Nigeria, in plant extraction and Ms. Wuraola Subuloye for her assistance in antioxidant assay.
REFERENCES


