

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

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I. INTRODUCTION

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 μ g/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 μ g/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.

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 peroxy 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. polita*, *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 Cameroun. 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 Ikire, 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. Qualitative 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, phlobatannins 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-Ciocalteu 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-Ciocalteu'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 NaNO_2 (0.5M) and 0.5 mL of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (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 ($y = 0.296x + 0.053$; $R^2 = 0.6469$) 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 ($y = 0.219x + 0.063$; $R^2 = 0.7460$) 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 K₄Fe(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 ($y = 0.917x + 0.398$; $R^2 = 0.6195$) [22], [23].

Total Alkaloid Determination: Alkaloid content of *F. sagittifolia* leaves and stem bark crude extracts 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:

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

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 Hex) 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 per 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
PHYTOCHEMICALS IN FSL AND FSS CRUDE EXTRACTS AND FRACTIONS

Phytochemicals	FSL HEX	FSS HEX	FSL EtOAc	FSS EtOAc	FSL crude	FSS crude	FSL Aq EtOH	FSS Aq EtOH
Terpenoids	present	present	present	present	present	present	present	present
Flavonoids	present	present	present	present	present	present	present	present
Alkaloids	present	present	present	present	present	present	present	present
Steroids	Absent	present	present	present	present	present	present	present
Cardiac glycoside	present	Absent	present	Absent	present	Absent	Absent	Absent
Phenolics	present	present	present	present	present	present	present	present
Tannins	present	present	present	present	present	present	present	present
Saponins	present	present	present	present	present	present	present	present
Anthraquinones	Absent	Absent	Absent	present	Absent	present	Absent	Absent
phlobatannins	Absent	Absent	present	Absent	present	Absent	present	present
Carbohydrate (Reducing sugar)	Absent	Absent	Absent	present	Absent	present	present	present

TABLE II
QUANTIFIED PHYTOCHEMICAL COMPOUNDS

Compounds	Total phenolics (GAE/g)	Total flavonoids (QUE/g)	Total Saponins (SA/g)	Total Tannins (TA/g)	Total Alkaloids (g)
FSL HEX	0.32±0.02	0.80±0.18	5.10±0.48	0.99±0.32	0.171±0.78
FSS HEX	0.47±0.05	0.37±0.08	3.95±0.07	1.31±0.02	0.058±0.14
FSL EtOAc	2.14±0.03	1.48±0.07	2.73±0.11	1.33±0.05	0.158±3.18
FSS EtOAc	2.22±0.11	1.20±0.01	2.59±0.46	0.99±0.31	0.168±1.27
FSL Crude	2.21±0.02	0.88±0.02	3.43±0.45	1.34±0.01	0.101±3.18
FSS Crude	3.53±0.03	1.84±0.04	2.81±0.48	1.26±0.07	0.050±0.71
FSL Aq EtOH	2.14±0.11	1.70±0.04	3.13±0.03	1.43±0.04	0.073±0.57
FSS Aq EtOH	3.25±0.03	3.41±0.08	2.42±0.23	1.52±0.05	0.070±0.49

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
ANTIOXIDANT ACTIVITY OF FSL AND STEM BARK CRUDE EXTRACTS AND FRACTIONS

SAMPLES	IC50 (µg/mL)
FSL Hex	243
FSS Hex	313.32
FSL EtOAc	79.837
FSS EtOAc	68.763
FSL Crude	84.732
FSS Crude	70.561
FSL Aq EtOH	63.092
FSS Aq EtOH	65.025
Vitamin C	21.239

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
TAC OF FSL AND STEM BARK CRUDE EXTRACTS AND FRACTIONS

SAMPLES	AAE (mg/g)
FSL HEX	18.03±0.81
FSS HEX	16.212±1.30
FSL EtOAc	103.788±1.53
FSS EtOAc	154.015±3.79
FSL crude	134.924±8.62
FSS crude	227.424±0.78
FSL Aq EtOH	134.243±2.47
FSS Aq EtOH	193.106±0.77

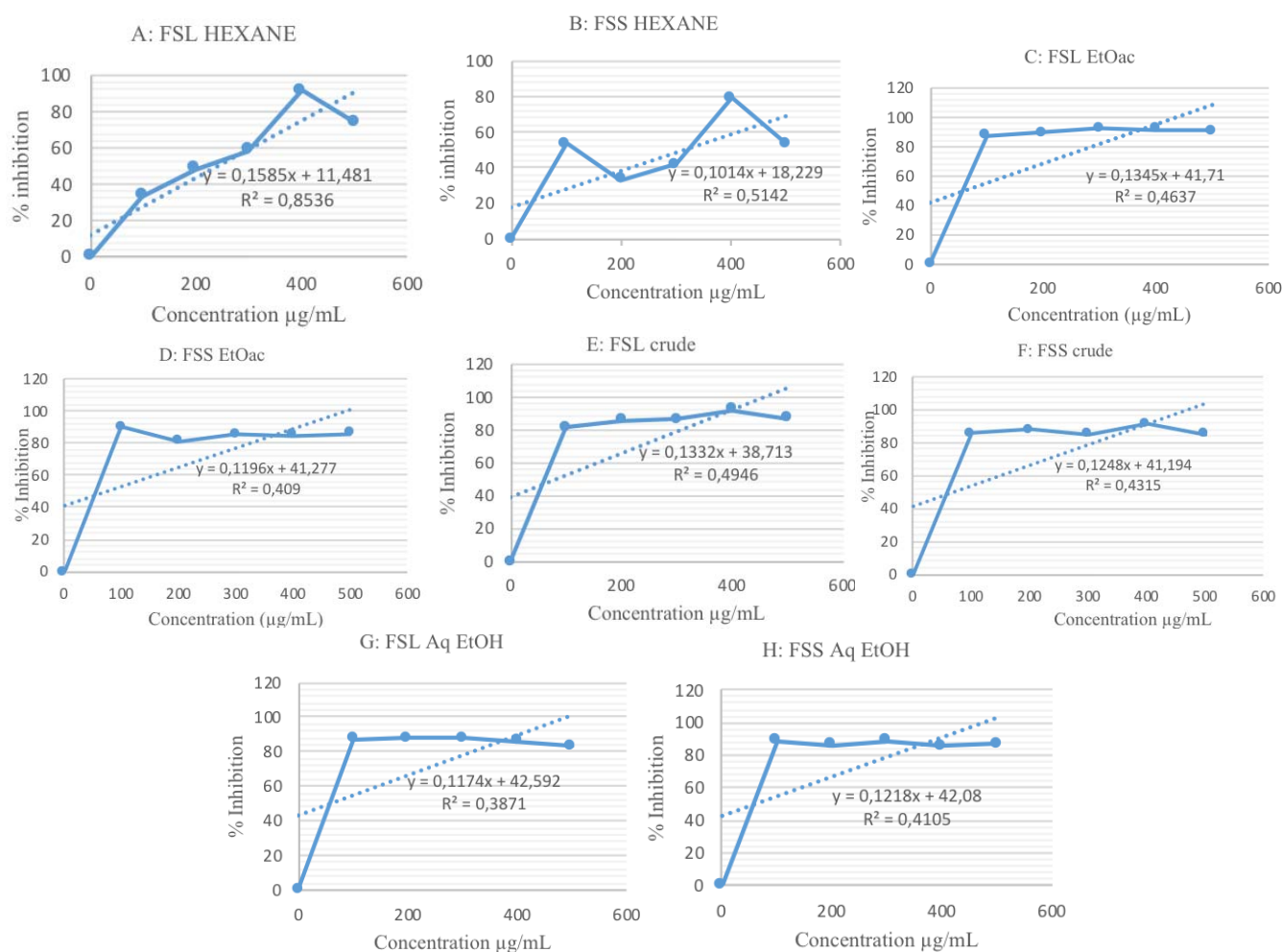


Fig. 1 IC₅₀ values obtained based on linear regression equation for *F. FSL* af and stem bark extracts and fractions: FSL- *Ficus sagittifolia* Leaf; FSS- *Ficus sagittifolia* Stem; HEX- hexane; ETOH- ethanol; Aq ETOH- aqueous ethanol

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.

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