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PHYTOCHEMICAL CONSTITUENTS AND *INVITRO* FREE RADICAL SCAVENGING ACTIVITIES OF METHANOL EXTRACT AND FRACTIONS OF *Ficus platyphylla* LEAVES (MORACEAE)

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ABSTRACT

The demand for plant-based antioxidants is expanding rapidly due to their effectiveness and accessibility. This study examined the phytochemical constituent and in vitro free radical scavenging activities of Methanol extract and fractions of *Ficus platyphylla* leaves. Standard known methods were used to determine the qualitative and quantitative phytochemical constituents and free radical scavenging activities of the extract and fractions of the plant dried leaves. Methanol extraction produced a yield of 12.64%. Qualitative phytochemical screening confirmed the presence of alkaloids in the extract and all but chloroform fraction. Flavonoids and steroids were present in all except n-Butanol fraction. Saponins were detected in only methanol extract and n-Butanol fraction while terpenoids were not present in n-Hexane, chloroform and n-Butanol fractions. Anthraquinones, coumarins, quinones and xanthoproteins were not confirmed in all fractions and extract. Quantitative analyses revealed a significant difference ($p < 0.05$) in the contents of the total phenolics and total flavonoids in the fractions when compared to the methanol extract. The leaf extract and fraction's ability to scavenge free radicals against 1,1-diphenyl-2-picrylhydrazyl (DPPH) demonstrated a concentration-dependent percentage inhibition. However, the methanol extract, n-Butanol and ethyl acetate fractions were most effective of all the fractions. This study has demonstrated the different fractions and methanol extract of *Ficus platyphylla* leaves can explored in the management of oxidative stress related complications in mammals.

Keywords: Antioxidants, Ascorbic Acid, DPPH, *Ficus platyphylla*, Free radicals, Phytochemicals

INTRODUCTION

The continuous search for natural plant products with antioxidant activities has gained tremendous attention in the biomedical research community overtime. Historically, medicinal plants have been and will continue to be a vital source of new and potent pharmaceuticals. The use of active chemicals derived from plants or their synthetic equivalents in medicine has become possible because of advancements in phytochemistry and pharmaceutical chemistry. (Dubale *et al.,* 2023). When compared to synthetic pharmaceuticals, most herbal drugs are considered to be safe and free of serious side effects (Shemishere *et al.,* 2019). In fact, according to statistics, 80% of people worldwide still rely on traditional medicine for their basic medical needs. The majority of this traditional medicine uses plant extracts or the active components of those extracts (WHO, 1993). Most of the medicinal properties of plants are harnessed in the form of phytochemicals. Phytochemicals are organic substances naturally present in plants, including vitamins, polyphenols, carotenoids, flavonoids, tannins, triterpenoids, steroids, saponins, and alkaloids (Zhang *et al.*, 2015). These substances have potent antioxidant and free radical scavenging properties that are crucial for both preventing and treating chronic illnesses (Ghosh *et al.,* 2013). Reactive oxygen species (ROS), another name for free radicals, have the capacity to harm cellular constituents, leading to illness. They play a role in the development of numerous human diseases, including cancer and cardiovascular conditions. etc. (Omoregie *et al.,* 2014; Ahmed *et al.,* 2015; Njoya, *et al.,* 2017).

For many years, the plant *F. platyphylla* has been used in Nigerian folk medicine to treat conditions like epilepsy, depression, psychosis, pain, and inflammation. The efficacy of this treatment is well known among rural communities in Northern Nigeria, where it is known locally as "Gamji" (Audu, 1989). The presence of saponins, flavonoids, and tannins in the methanol extract of F. platyphylla stem bark was discovered using high performance liquid chromatography (HPLC) and preliminary phytochemical screening (Chindo *et al.,* 2008). Previous investigations have shown that the methanol extract of the stem bark of F. platyphylla has analgesic and anti-inflammatory properties in a variety of tests, including those that cause acetic acid-induced writhing, formalin-induced nociception, and albumin-induced oedema in mice (Chindo *et al.,* 2016). The standardised extract of *F. platyphylla* stem bark has also been shown to have anticonvulsant and behavioural effects (Chindo *et al*., 2014). Oral administration of the methanol extract of F. platyphylla stem bark and leaves is safe for rodents at dosages up to 3000 mg/kg body weight, according to sub-chronic toxicity tests (Chindo *et al.,* 2012; Ukwani-Kwaja *et al.*, 2021). According to past toxicological research, F. platyphylla has several bioactive components such tannins, saponins, and flavonoids that have the ability to reduce inflammation and are generally harmless. .However, there this paucity of reports on the phytochemical constituents and free radical scavenging activities of the methanol extracts and fractions of the leaves. Hence this study is aimed at providing information for the aforementioned.

MATERIALS AND METHODS

Collection, Extraction and Fractionation of *Ficus platyphylla* leaves

The plant's leaves were gathered from bushes in the Ungwar jeji village in Kebbi State. The plant was given the voucher number FBK-H-60 after being botanically identified and authenticated by a taxonomist at the herbarium section of the Department of Biological Sciences, Federal University Birnin Kebbi. Before usage, the authenticated plant samples were air-dried, ground, and stored in an airtight container. Methanol extracts of the leaves were prepared by soaking a weighed quantity (300 g) of the crushed sample in a specific

volume (2.5 litres) of methanol for 72 hours and then filtered using Muslin cloth. The extracts were concentrated by using a rotary evaporator at 40°C and further freeze-dried. Following that, the dried extract was divided into fractions using a separator funnel and solvents in ascending polarity order. In order to separate the Ficus platyphylla leaf methanol extracts into hexane, chloroform, ethyl acetate, and n-butanol, 500 mL of distilled water was used. At the proper temperatures, all derived fractions were concentrated using rotary evaporators, and they were then freeze-dried before being kept in the refrigerator until they were needed.

Qualitative Phytochemical Screening
Phytochemicals in the study plants were screened and identified chemically in the extracts using protocols previously reported by Shemishere *et al.* (2020).

Test for Flavonoids
A test tube containing 2 mL of extract solution, 5 mL of diluted ammonia, and 1 mL of concentrated H_2SO_4 was combined. A golden hue indicated the presence of flavonoids.

Test for Tannins
A test tube containing one millilitre (1 mL) of extract (filtrate) was heated to boiling for five minutes. After adding a few drops of 15% ferric chloride, the presence of tannins was confirmed by the blue-black colouring**.**

Test for Cardiac glycosides
Using Killaini's test, the cardiac glycoside test was conducted. A test tube containing one millilitre of extract was combined with two millilitres of glacial acetic acid, one drop of 15% ferric chloride, and one millilitre of strong sulfuric acid. The presence of cardiac glycosides was detected by a brown coloration that formed at the contact.

Test for Saponins
As a screening test for saponins, the capacity of saponins to cause frothing in aqueous solution was employed. Five milliliters of distilled water were added to 1 mL of extract. The liquid was vigorously stirred, and observed for frothing.

Test for Steroids
In a test tube, 1 mL of extracts was combined with 2 mL of concentrated H_2SO_4 and acetic acid. Steroid presence was indicated by a shift from violet to blue-green colouring.

Test for Terpenoids
Salkowski's test was used to determine the presence of terpenoids. To 1 mL of the extract in a test tube, 2 mL chloroform was added, along with 3 mL of concentrated H_2SO_4. A reddish-brown coloration at the interface indicated the presence of terpenoids.

Test for Alkaloids
The presence of an alkaloid was determined by the appearance of a cream-colored precipitate in the Mayer's test, which involved combining 1 mL of extracts with 3 drops of the Mayer's reagent and bringing the solution up to 100 mL with distilled water.

Test for Anthraquinones
Magnesium acetate was diluted with a few drops and added to the test sample. The production of pink coloration was a sign that anthraquinones were present.

Test for Phenols
In a test tube containing 2mL of sample, a few drops of 10% aqueous FeCl3 solution were added. Phenols were present when bluish-green or red colours formed.

Test for Coumarins
Two millilitres of the extract solution received a few drops of alcoholic NaOH. The existence of coumarin was established by the colour yellow.

Test for Quinones
Concentrated NaOH was added to 2mL of the test sample, and the presence of quinones was detected by the appearance of colour.

Test for Xanthoproteins
A few drops of pure HNO_3 were added to 1 ml of the test sample and thoroughly mixed. 2 ml of the ammonia solution were then added. The presence of xanthoprotein was revealed by the production of red precipitate.

Test for Fixed Oil
The sample was sandwiched between two filter papers in a small amount. Grease stains showed that there were fixed.

Total Phenolics Determination
The total phenolic content was calculated using the Folin-Ciocalteau method, as described by Cicco *et al*. (2009) and Shemishere *et al.* (2020). The Folin-Ciocalteau reagent (phosphomolybdate and phosphotungstate) reduction by phenolic chemicals is the basis for this experiment. A spectrophotometer can detect the decreased Folin-Ciocalteau reagent at 760 nm since it is blue in colour. One mg/mL quantities of gallic acid or extracts/fractions were created in DMSO. In 4.5 mL of distilled water, 0.5 mL of the extract/fraction and 0.5 mL of a Folin Ciocalteau reagent that had been diluted 10 times were mixed. The tubes were then refilled with 5mL of 7% sodium carbonate and 2mL of distilled water. At 760 nm, the mixture's absorbance was measured after standing at room temperature for 90 minutes. Gallic acid was used as the positive control throughout all determinations, which were carried out in triplicates. Gallic Acid Equivalent (GAE)/g of extract/fraction was used to measure the overall phenolic content.

Total Flavonoids Determination
The Miliauskas *et al.* (2004) technique reported by Shemishere *et al.* 2019 was used to calculate the total flavonoid content. Two millilitres 2% $AlCl_3$ in ethanol and 2 mL of methanol were mixed, and the standard values ranged from 0.1 to 1.0 mg/mL. The extract/fractions at a concentration of 2ml of 1mg/ml and the 2% $AlCl_3$ in ethanol were also added. The absorbance at 420 nm was measured following an hour of incubation at room temperature. Comparable quantities of quercetin, a positive control, were used. Quercetin equivalent (QE) mg/g of extract or fraction was used to calculate the quantity of flavonoids present.

Estimation of Diphenyl-2-Picryl-Hydrazyl (DPPH) Radical Scavenging Activity
A slightly modified version of Brand Williams et al. (1995) method was used to assess the leaf extracts' capacity to scavenge free radicals against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The assay is based on the antioxidant compounds' ability to reduce DPPH by donation of hydrogen, which causes a change in colour from deep violet to golden yellow, which was measured with a spectrophotometer at 517 nm. In this, 2 mL of various

concentrations (0.2 - 1.0 mg/mL) of the standard, extracts, or fractions were added to 0.5 mL of a 0.3 mM DPPH solution in methanol. After 15 minutes of shaking and incubation at room temperature in the dark, the reaction tubes' absorbance was measured at 517 nm. Every test was run in triplicate. Ascorbic acid was produced in test samples' concentrations and used as a standard control. In order to simulate the test samples, a blank containing 0.5 mL of 0.3 mM DPPH and 2 mL methanol was created. The following formula was used to compute the radical scavenging activity.

DPPH radical scavenging activity (%) = $[\frac{(A0-A1)}{A0}]\ x\ 100$

where A0 was the absorbance of DPPH radical + methanol; A1 was the absorbance of DPPH radical + sample extract/ fraction or standard.

Statistical Analysis

The average and Standard Error of Mean (SEM) of three replicates were used to express the experimental results, and P values < 0.05 were accepted as significant. The data were subjected to one-way analysis of variance (ANOVA) when necessary, and using the IBM SPSS statistical tool, differences between samples were determined using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Table 1: Result of the qualitative phytochemical screening of methanol extract and fractions of *Ficus platyphylla* leaves

Phytochemicals	Methanol	n-Hexane	Ethyl Acetate	Chloroform	n-Butanol
Alkaloids	+	+	+	-	+
Anthraquinones	-	-	-	-	-
Cardiac glycosides	++	-	+	+	+
Coumarin	-	-	-	-	-
Fixed Oil	+	+	-	-	-
Flavonoids	++	+	+	++	-
Phenol	+	+	+	++	+
Quinones	-	-	-	-	-
Saponins	++	-	-	-	+
Steroids	+	+	+	+	-
Tannins	++	-	+	-	-
Terpenoids	+++	-	+	+	-
Xanthoproteins	-	-	-	-	-

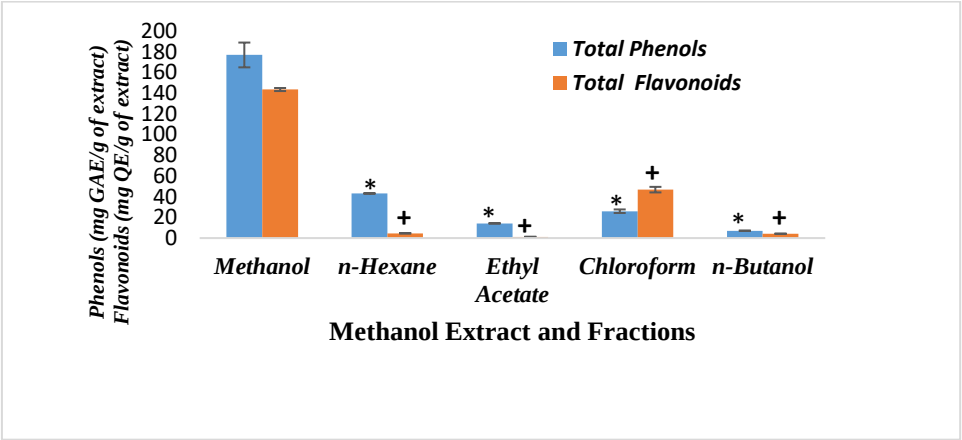

Figure 1: Total phenols and total flavonoids contents of *Ficus platyphylla* leaf Methanol extracts and its fractions from n-Hexane, Ethyl acetate, Chloroform and n-butanol. Bars with "*" and "+" on their top indicate significant difference ($p < 0.05$) when compared to the values of the methanol extracts respectively for total phenol and total flavonoids.

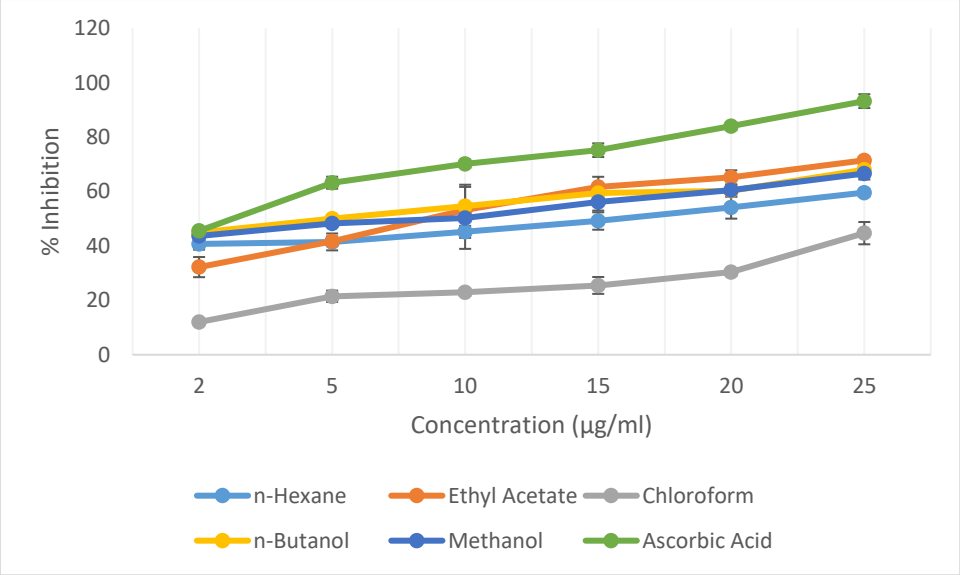

Figure 2: DPPH Scavenging activity of *Ficus platyphylla* leaf Methanol extracts and its fractions from n-Hexane, Ethyl acetate, Chloroform and n-butanol.

Discussion

Phytochemical analysis can identify specific groups of substances in a sample based on observations such as colour changes or precipitate development (Dubale *et al.,* 2023). The initial stage in a research strategy targeted at the separation and purification of natural compounds with bioactivities is often screening plant materials for their phytochemicals (Omoregie and Oikeh, 2015). It gives an indication of the potential uses of the plant material by enabling the researcher to quickly identify the phytochemicals present in a plant material (Oikeh *et al.,* 2013). The results of the qualitative phytochemical shown in Table 1 revealed the presence of phytochemicals across the fractions and extract. We determined that all but the chloroform fraction of the extract contained alkaloids. All save the n-Butanol fraction contained flavonoids and steroids. Terpenoids were not found in the n-Hexane, chloroform, or n-Butanol fractions, while saponins were only found in the methanol extract and n-Butanol fraction. Saponins were detected in only the methanol extract and n-butanol fraction. In all the fractions and extract, anthraquinones, coumarins, quinones, and xanthoproteins were not detected. Sheidu *et al*. (2020) and Magili and Agber *et al.* (2021) has shown the presence of these phytochemicals in the methanol stembark and ethanol and aqueous leaves extract of *Ficus platyphylla*. Some of these components are thought to be active secondary metabolites that are in charge of the significant pharmacological effects of medicinal plants (Mohammed *et al.*, 2017). The presence of alkaloids suggests that the leaves have analgesic, anti-inflammatory, and increased disease resistance and stress tolerance abilities (Taiwo *et al.*, 2017). Terpenoids are utilised in medicine and have biological effects. For instance, it has been discovered that several terpenoids have anti-inflammatory, anti-microbial, and anti-cancer properties (Singh and Sharma 2015). Results of the total phenolic and total flavonoid analysis displayed in figure 1 showed that the methanol extracts differed significantly ($p < 0.05$) from the other fractions. In the plant kingdom, phenolics, which are highly diverse, make up the bulk of secondary metabolites. Plant polyphenols have gained growing attention as a result of their powerful antioxidant abilities and their notable contributions to the prevention of several oxidative stress-related illnesses, including cancer (Dai and Mumper 2010). The majority of plants include a group of polyphenolic compounds known as flavonoids. They are useful in the prevention and treatment of various ailments because they are known to exhibit a variety of therapeutic properties (Ayoola *et al.,* 2008). The most prevalent and widely distributed classes of plant phenolics are flavonoids (Omoregie *et al.,* 2014). They are potent, water-soluble super antioxidants that work to chelate transition metals, scavenge free radicals, and prevent peroxidation (Flora, 2009; Oseni and Okoye, 2013). Antioxidants are reducing substances that limit oxidative damage to biological structures by giving free radicals electrons and passivating them. Free radicals are intimately related with oxidative damage. (Herrling *et al.,* 2008). The percentage DPPH free radical inhibition depicted in figure 2 revealed that the extract and fractions showed antioxidant activities with increased concentration. The existence of the variety of phytochemicals that were found through their screening as demonstrated in other studies can be inferred from the observed free radical scavenging activity of our extract and fractions as demonstrated in the DPPH assay as compared to that of the standard, ascorbic acid. (Sharida *et al.,* 2012; Yan *et al.,* 2009; Shemishere *et al.,* 2020)

CONCLUSION

This research work has revealed that the screened phytochemicals are unevenly distributed amongst the n-hexane, ethyl acetate, chloroform and n-butanol fractions of the methanol extract of the leaves of *Ficus platyphylla*. The quantity of the phenolics and flavonoids are more in the methanol extract than in the fractions, However, amongst the fractions, the n-hexane fraction had the highest content of phenolics while the chloroform fraction had the largest content of flavonoids. All fractions and extract scavenged DPPH free radical in a concentration dependent manner. Nevertheless, the presences of the diverse phytochemicals might be attributed to this capacity. Further studies are suggested to explore the specific phyto-compounds in these fractions in order to harness them for the management of oxidative stress related complications.

CONFLICT OF INTEREST

The Authors declare no conflict of Interest

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