PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY STUDIES OF THE ETHANOL LEAF EXTRACT OF FICUS SYCOMORUS L. (FAMILY: MORACEAE)


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ABSTRACT

Ficus syncomorus (Sycamore fig.) Family Moraceae is a plant used in African traditional medicine to treat mental illness, dysentery, cough, diarrhea, tuberculosis and cancer. The crude ethanol extract, n-Hexane and ethyl acetate fractions of the leaves of the plant were subjected to preliminary phytochemical screening using standard procedure as well as qualitative and quantitative antioxidant activity studies using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) assay. The results of phytochemical screening showed that the crude ethanol extract contains alkaloids, flavonoids, saponins, tannins, terpenoids and anthraquinones, the n-hexane fraction contains terpenoids, alkaloids and anthraquinones while the ethylacetate fraction contains alkaloids, saponins, tannins, terpenoids, flavonoids, anthraquinones and cardiac glycosides coumarins were found to be absent in the leaves of the plant. The results of the antioxidant activity studies revealed that Ficus syncomorus leaf extract has IC50 of 44.83µg/ml, 58.46.07µg/ml and 42.00µg/ml for crude ethanol extract, n-hexane and ethylacetate fractions respectively. Vitamin C which is the standard drug used was found to have IC50 of 25.00µg/ml. The plant exhibited antioxidant potential and could be useful in diseases involving oxidative stress such as age related cancers, stroke, Rheumatoid, arthritis and heart diseases.

Keywords: Antioxidant; Ficus syncomorus; Moraceae

INTRODUCTION

Ficus is the Latin form of fig, derived from the Persian ‘fica’. In Greek ‘syka’ means fig. The name of the species comes from the Greek ‘sykamorea’ (sycamore), used in the Gospel according to St. Luke; it was the tree that Jesus cursed because it was barren (Hyde et al., 2013). Ficus syncomorus is a large, semi-deciduous spreading savannah tree, up to 21 (max. 46) m; it is occasionally buttressed. Its leaves are broadly ovate or elliptic, the sub base is cordate, apex is rounded or obtuse and is scabrous above; petiole is 1-5 cm long, with five to seven pairs of yellow lateral veins; lowest pair originates at the leaf base. The plant is widely distributed in tropical Africa stretching from Senegal to South Africa, Nigeria, Niger, Mali, South Africa, Guinea, Kenya, Tanzania, Somalia, Ethiopia and Ivory Coast. In Nigeria the plant is mostly found in semi-arid regions (Williams et al., 1980).

The plant is referred to by number of local names such as Sycamore fig (English), Baure (Hausa, Northern Nigeria), Tarmu (Kanuri), and Kamda (Babur/Bura), among others (Hyde et al., 2013).

Ficus sycomorus is used traditionally in the treatment of snake bites, jaundice, chest pains, dysentery, cool, coughs and throat...
infections (Sofowora, 1993). In northern Nigeria, the stem bark of *Ficus sycomorus* is used traditionally to treat fungal infections, jaundice and dysentery (Berg and Corner, 2005). The Hausa and Fulani tribes of northern Nigeria use the stem-bark of *F. sycomorus* to treat diabetes mellitus, fungal infection, jaundice and dysentery (Hassan et al., 2007; Aduom et al., 2012). The parts of *F. sycomorus* used traditionally for the treatment of tumors and diseases associated or characterized by inflammation include the fruits in different stages of ripening, fresh or dry, tree bark, leaves, twigs and young shoots, and also latex from the bark, fruit and young branches (Lansky et al., 2008).

**MATERIALS AND METHODS**

**Collection, and Identification, of plant materials**

The leaves of *Ficus sycomorus* were collected in April, 2013, at the fields of Ahmadu Bello University Zaria, Nigeria. The plant was identified by Mallam U.S. Gallah of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria, (voucher specimen number 1466). The plant material was air dried under shade and size reduced manually using clean mortar and pestle.

**Preparation of the extract**

2.5 Kg of the plant material was subjected to cold maceration using 75% ethanol for 24 hours. The extract was filtered using Whatman filter paper (No. 1) and the filtrate evaporated under reduced pressure and dried to afford 18.20 % (w/w) of a brownish residue referred to as crude ethanol extract. The crude ethanol extract was further fractionated successively using n-hexane, chloroform, ethylacetate and n-butanol. The crude ethanol extract, n-hexane fraction and ethylacetate fraction were used for this research.

**Phytochemical screening**

Preliminary qualitative phytochemical tests were carried out on Crude Ethanol Extract, n- hexane and ethylacetate fractions employing standard procedures (Sofowora, 1982).

**Antioxidant studies**

**Qualitative antioxidant studies**

The crude extract and the various fractions were spotted on a TLC plate and developed using hexane-ethylacetate mixture at the ratio of 8:2 as a solvent system in a chromatographic tank. The developed chromatogram was sprayed with 0.15% w/v DPPH in methanol solution using an atomizer. The plate was observed for the presence of yellow colour on a pinkish/purple background on the TLC plate which is an indication for the presence of antioxidant principles (Saha et al., 2008).

**Quantitative antioxidant studies**

The free radical scavenging activity of the leaves of *Ficus sycomorus* was determined using DPPH (Braca et al., 2001). 0.004% w/v DPPH solution was prepared in 95% methanol. To 1ml of various concentrations of the extract (20, 40, 60, 80, and 100µg/ml) 2ml of DPPH solution was added. An equal amount of methanol and DPPH served as control. The mixture was shaken vigorously and was left to stand in dark for 30mins. The absorbances of the resulting solutions were measured using UV spectrophotometer at 520nm. The experiment was performed in triplicate and the percentage scavenging activity of each extract on DPPH radical was calculated as follows.

\[
\text{Scavenging activity (\%)} = \left(\frac{A_b - A_e}{A_b}\right) \times 100
\]

Where; \(A_b\) = absorbance of blank sample

\(A_e\) = absorbance of the solution containing the extract
The IC_{50} (concentrations of the extracts that inhibit 50% of the free radicals) were calculated from the graph of scavenging activity plotted against sample concentrations using Microsoft excel software (Viturro et al., 1999).

RESULTS

Phytochemical screening

The result of Phytochemical screening of the Crude ethanol extract, n-hexane and Ethylacetate fractions of the leaves of *F. sycomorus* revealed that the crude ethanol extract contains alkaloids, saponins, terpenoids, flavonoids, tannins, cardiac glycosides and anthraquinones; the N-hexane fraction contains alkaloids, terpenoids and cardiac glycosides while ethylacetate fraction contains Alkaloids, Saponins, Tannins, Flavonoids, Anthraquinones and Cardiac glycosides (Table I).

Table I: Results of phytochemical analysis of leaves of *F. sycomorus*

<table>
<thead>
<tr>
<th>Phytochemical constituent</th>
<th>CEE</th>
<th>NHF</th>
<th>EAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Keys: + = Present; - = Absent; CEE = Crude ethanol Extract; EAF = Ethylacetate fraction; NHF = n-hexane Fraction
RESULTS OF ANTIOXIDANT ACTIVITY STUDY

Qualitative studies
The qualitative antioxidant activity study of the Crude ethanol extract, N-hexane and ethylacetate fractions using different systems shows that the plant possesses antioxidant activity due to the appearance of a yellow spot on a purple background as shown on plate 1.

Quantitative study
The result of in vitro DPPH radical scavenging activity of the crude ethanol plant extract, n-hexane and ethylacetate fractions showed an increase in antioxidant activity with increase in concentration of extract. The linear graph (figure 1) showed good association between the antioxidant activity and concentration of the plant extracts as the correlation coefficient tends toward one.

Plate 1: Qualitative antioxidant activity studies of crude ethanol extract (A), n-hexane fraction (B), ethyl acetate fraction (C) using hexane-ethyl acetate (8:2) as solvent system
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Figure. 1: A graph % inhibition against concentration of crude ethanolic extract and n-hexane fraction the of leaf of *Ficus sycomorus*

Table II: IC\textsubscript{50} values of th ecrude ethanol extract, n- hexane fraction and ethylacetate fraction of leaf extract *Ficus sycomorus*

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>IC\textsubscript{50} (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Ethanolic extract</td>
<td>44.83</td>
</tr>
<tr>
<td>Hexane Fraction</td>
<td>58.46</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>42.00</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>25.00</td>
</tr>
</tbody>
</table>
DISCUSSION

The results of phytochemical analysis revealed that the crude ethanolic extract of *Ficus sycomorus* leaves contains flavonoids, alkaloids, tannins, saponins, cardiac glycosides, anthraquinones and terpenoids; while the N-hexane fraction contains alkaloids, terpenoids and cardiac glycosides.

Flavonoids are most commonly known for their antioxidant activity; they are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants (Spencer et al., 2008). Flavonoids have been reported to have anti-viral, anti-inflammatory, antitumor and antioxidant activities while tannins have been reported to be active against ulcerated and inflammed tissue as well as possessing antioxidant activity (Subhuti, 2003). Alkaloids are known to have antimalarial, analgesic and even antitumor activities. Cardiac glycosides are known to improve cardiac activities (Syke, 2011).

Free radicals are easily formed when a covalent molecular bond is broken and one electron remains with each newly formed atom. When 100μg/ml of the extract was spotted on a TLC Plate, the result obtained when a chromatogram was sprayed with DPPH, was a change of colour (yellowish) colour development on pinkish back ground on the TLC plate is an indicator for the presence of antioxidant substance which constitute the qualitative analysis.

The free radical scavenging activity of *Ficus sycomorus* evaluated using DPPH method is presented in table 2 and table 3. The model of DPPH free radical scavenging activity can be used to evaluate the antioxidative activity in relatively short time. The absorbance decreases as a result of a colour change from purple to yellow as the radical is scavenged by antioxidants through donation of hydrogen to form a stable free radical DPPH molecule (Williams et al., 2003).

![Figure. 2: A graph % inhibition against concentration of Vitamin C](image_url)
On addition of 2mL of DPPH solution to the various concentrations of the prepared crude ethanol, n-hexane and ethylacetate fractions of the leaf extract of *Ficus sycomorus*, the purple colour of DPPH was altered and as concentration decreases, the purple colour of DPPH gradually reappears although in the least concentration (20μg/mL), which increases with the intensity of the DPPH. The colour observed when DPPH was added to the plant extract was yellowish grey, although yellow colour is the expected colour, but due to complex nature of our plant extract, it contains a diverse class of constituent unlike the vitamin C. The yellowish grey is intermediate between the original colour of the extract and the expected yellow colour of the resultant solution, the appearance of yellow colour in the colourless solution of vitamin C on addition of DPPH signifies the scavenging of free radicals of DPPH hence antioxidant property. As mentioned above, these plants contain flavonoids saponins, alkaloids and terpenoids. The antioxidative effect is mainly due to phenolic components, such as flavonoids and phenolic diterpenes (Shahidi et al., 1992). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994).

From this observation, it can be inferred that the crude ethanol and n-hexane fractions of leaf of *Ficus sycomorus* have some degree of antioxidant property.

References


