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RESEARCH ARTICLE

# Evaluation of HPLC-UV-DAD and antiproliferative characteristics of the leaf infusion of *Ximenia americana* Linn.

**Samuel Ehiabhi Okhale[1]*, Amarachi Clare Nnachor[1,2], Udeme Effiong Bassey[1]**

[1] Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, Idu Industrial Area, P.M.B. 21 Garki, Abuja, Nigeria
[2] Department of Pure and Applied Chemistry, Nnamdi Azikiwe University, P.M.B 5025, Awka, Anambra State, Nigeria
*Corresponding Author: Dr. Samuel Ehiabhi Okhale; Tel: +2348036086812;
E-mail: samuelokhale@gmail.com

**ABSTRACT**

*Ximenia americana* (*X. americana*) is reputable for the treatment of various ailments in Nigeria. The hot aqueous extract of *X. americana* leaf (XA) was obtained by infusion. The antiproliferative potential of XA was evaluated employing *Sorghum bicolor* seed radicle as test subject over the period of 48-96 hours. The mean radicle lengths (mm), percentage inhibition and percentage growth were determined. XA was chemically characterized using colour reactions and high performance liquid chromatography with UV-diode array detector (HPLC-UV-DAD). Phytochemical investigation indicated the presence of tannins, saponins and flavonoids. HPLC analysis revealed thirteen peaks with rutin and ferullic acid eluting at 6.886 and 7.796 minute respectively. XA significantly ($p < 0.0001$) inhibited *S. bicolor* seed growth over a period of 48-96 h against the control seeds. At 96 h, XA dose-dependently inhibited seed growth, giving percentage inhibition of 23.24, 29.06, 30.68, 38.27, 49.57, 50.39, 64.60, 79.67 and 82.01% for seeds treated with 1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml 16 mg/ml, 24 mg/ml, 32 mg/ml, 40 mg/ml and 48 mg/ml respectively with $IC_{50}$ of 24 mg/ml. Methotrexate 0.167 mg/ml used as positive control gave inhibition of 92.76% at 96 h. This result revealed the potential of XA to inhibit the growth of fast proliferating cells of *S. bicolor* seed radicle.

**Keywords:** *Ximenia americana*; Antiproliferative; *Sorghum bicolor*; Caffeic acid; Rutin.



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## INTRODUCTION

Cancer generally refers to a group of diseases that cause cells in the body to change and grow out of control. It can spread to other parts of the body through lymph and blood. Breast cancer has been described as the most commonly diagnosed malignancy and the leading cause of cancer-related deaths of women worldwide [1]. Incidence of cancer is on the increase worldwide, with estimated 14.1 million new cancer cases in 2012; female

breast, colorectal and stomach cancers accounted for over 40% of all cases diagnosed globally. Lung cancer accounted for 16.7% of all new cases in men [2].

*Ximenia americana* belongs to the family Olacaceae. The genus *Ximenia* has eight different species namely *Ximenia roiigi, Ximenia aegyptiaca, Ximenia parviflora, Ximenia coriaceae, Ximenia aculeate, Ximenia caffra, Ximenia americana and Ximenia aegyptica* It is commonly referred to as wild plum and tallow nut. It is a shrub or small tree that grows to about 7 m high, with zigzag branches. The bark is black or grey-brown, smooth when young but becomes rough with age having stiff axillary spines. The leaves are alternate or clustered on spur shoots. The seed morphology is variable. Flowers are green white to greenish yellow, scented and 5-10 mm long in small branched inflorescences [3]. Native names in Nigeria: Hausa (Tsada), Fulani (Chabbull), Tiv ( Anomadze) ,Yoruba (Igo) and Benin (Alimo-mamiwota) [4].

The methanolic extract of leaves of *Ximenia americana* has been proven to have anti-diabetic effect in rats [5]. The ethanolic extract of the bark of *X. americana* revealed the presence of the following metabolites; flavonoids, steroids, tannins, alkaloids, phenolic compounds, saponin, terpenoids, and glycosides. It also has activity against *Staphylococcus aureus* and low activity against *Pseudomonas aeruginosa*. Other investigations led to isolation of two compounds. The first one which is new is 3-methyl-1-oxoisochroman-8-carboxylic acid and the second compound which is a known steroid is ergosta-4,6,8,22-tetraen-3-one [6].

The plant has also been reported to have anti-HIV/AIDS related diseases effects such as abscesses, skin rashes, diarrhea and gonorrhea. The anti-HIV/AIDS effect of *Ximenia americana* is due to the fact that it contains oleic, ximenic (hexacos-17-enoic), linoleic, linoleric and stearic acids. Its oil consists of very long chain fatty acids with up to 40 carbon atoms used to manage STIs including gonorrhoea [7].

Literature review reveals that the plant *Ximenia americana* has been alleged to have antineoplastic activities, antimicrobial and anti-inflammatory activities and lots more. The aqueous plant extract of the leaves is used in the treatment of cancer in African traditional medicine [8]. *X. Americana* has a wild varieties of phytochemical compounds which among them are the following secondary metabolites; saponins, flavonoids, tannins, terpenoids, sterols, quinines, alkaloids, cyanogenetics glycosides, cardiac glycosides and carbohydrates in the form of sugars and soluble starch [10]. The essential oils of the leaves of *X. americana* using GC-MS and identified 33 components representing 98% of the total oil. The main constituents analysed are benzaldehyde 63.5%, hydroxybenzylcyanide 13%, isophorone 3.5%. The hydroxybenzylcyanide is known as a primary breakdown products of glucosinolates found mainly in the Brassicaceae family [11]. The fixed oil present in the seeds of *X. americana* [12].

The ethanolic extract was reported to contain polyphenols, cyanogenic acid, glycoside sambunigrin, gallic acid, gallotannins-β-glucopyranose, flavonoids, quercetin, quercitrin (quercetin3-0-α-rhamnopyranoside), avicularin (quercetin-3-0-α-arabinofuranoside), Quercetin-3-0-β-xylopyranoside, quercetin-3-0-(6-galloyl)-β-glycopyranoside. The flavonoids were active both as enzymes inhibitors and DPPH radical scavengers [13]. The aim of this study is to evaluate the phytochemical, high performance liquid chromatography profile and antiproferative activity of the leaves of *Ximenia americana*, a reputable herbal medicine grown in Nigeria.

## MATERIALS AND METHODS

### Chemicals and reagents

Unless otherwise stated, all chemicals and reagents were of analytical grade and purchased from Sigma Aldrich (Germany). All the solvents for chromatographic purpose were HPLC grade, purchased from Sigma Germany.

### Experimental plant

The leaves of *Ximenia americana* used for this study were collected from Bamburu-Gwagwalada Abuja by Mallam Muazzam of the National institute for Pharmaceutical Research and Development Idu Abuja, Nigeria.

It was identified and authenticated by Mr. Akeem Lateef at the Herbarium of the National Institute for Pharmaceutical Research and Development Idu Industrial Area Abuja.

**Preparation of plant extract**

The fresh leaves of *Ximenia americana* were air-dried at room temperature. The dried sample was pulverized. Then 50 g of powdered sample was weighed and extracted by hot water (1000 ml) infusion in an air tight container for 24 h. The resultant mixture was filtered using a funnel whose exit was tightened with cotton wool. The filtrate was dried over a water bath to yield *Ximenia americana leaves* aqueous extract [14].

**Phytochemical analysis**

Phytochemical screening was conducted on the aqueous extract for secondary metabolites and the following was present; carbohydrates, tannins, saponins and flavonoids [15].

**High performance liquid chromatography analysis**

The bioactive constituents of XA was analysed by high performance liquid chromatography (HPLC) with UV diode array detector (UV-DAD). The HPLC consisted of Ultra-Fast LC-20AB equipped with SIL-20AC auto-sampler; DGU-20A3 degasser; SPD-M20A UV-diode array detector; column oven CTO-20AC, system controller CBM-20Alite and Windows LC solution software (Shimadzu Corporation, Kyoto Japan); column, 5µm VP-ODS $C_{18}$ and dimensions (4.6 x 150 mm). The chromatographic conditions included mobile phase: 0.2% v/v formic acid and acetonitrile (20:80); mode: isocratic; flow rate 0.6 ml/min; injection volume 10 µl of 100 mg/ml solution of extract in water; detection UV 254 nm. The HPLC operating conditions were programmed to give solvent B: 20%. Column oven temperature was 40 $^{o}$C. The total run time was 30 minutes. Flavonoids and phenolic acid standards such as apigenin, rutin, quercetin, caffeic acid, ferulic acid were employed for the identification of the phytoconstituents of XA by comparing the retention time under similar experimental conditions [16]

**Determination of growth inhibitory effect**

The modified methods of Ayinde et al. [17] and Chinedu et al. [18] were used for this study. *Ximenia americana* hot aqueous extract (300 mg) was dissolved in 60 ml of distilled water to obtained 50 mg/ml stock solution. Various concentrations (1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml 16 mg/ml 24 mg/ml 32 mg/ml 40 mg/ml and 48 mg/ml) of the extract were prepared. Methotrexate was made to a concentration of 0.167 mg/ml as positive control. Petri dishes were layered with cotton wool and filter paper (Whatman No. 1). Twenty seeds (n = 20) of *S. bicolor* were placed in each of the Petri dishes. The control seeds were treated with 15 ml distilled water. The test seeds were treated with the different preparations of the extract as the seeds in each specific Petri dish received 15 ml of a particular concentration (the seeds in the eleven different Petri dish were treated with 1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml, 16 mg/ml, 24 mg/ml, 32 mg/ml, 40 mg/ml and 48 mg/ml concentration respectively. The seeds were incubated in a dark room and observed for growth after 24 h. The mean lengths (mm) of radicle emerging from the seeds were measured after 48, 72 and 92 h. The percentage inhibition was calculated as [(mean radicle length control – mean radicle length treated)/mean radicle length control] ×100. Percentage growth was calculated as 100 – % inhibition. Percentage inhibition and percentage growth at 48, 72 and 92 h for seeds treated with 1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml, 16 mg/ml, 24 mg/ml, 32 mg/ml, 40 mg/ml 48 mg/ml, negative control and the positive control methotrexate at 0.167 mg/ml are as shown in Table 1.

### Statistical Analysis

The data obtained were expressed as mean ± standard error of mean and analyzed using Graph pad prism (version 7). Two way analysis of variance was used to test for significance. $P<0.0001$ was considered to be significant.

## RESULTS

### Phytochemical analysis

Extraction of 50 g of *Ximenia americana* powdered sample by hot water infusion yielded 5.6 g (11.2%) of the dried extract. Phytochemical screening indicated the presence of tannins, saponins, flavonoids, and carbohydrates.

### High performance liquid chromatography analysis

The HPLC chromatogram of XA shown in Figure 1, showed that thirteen peaks were detected as the constituents with retention times in minutes of 3.530, 4.070, 5.896, 6.886, 7.796, 10.767, 11.823, 13.790, 15.431, 17.898, 18.928, 22.019 and 25.752. Compounds with retention time in minute of 6.886 and 7.796 minute corresponded to rutin and ferullic acid respectively.

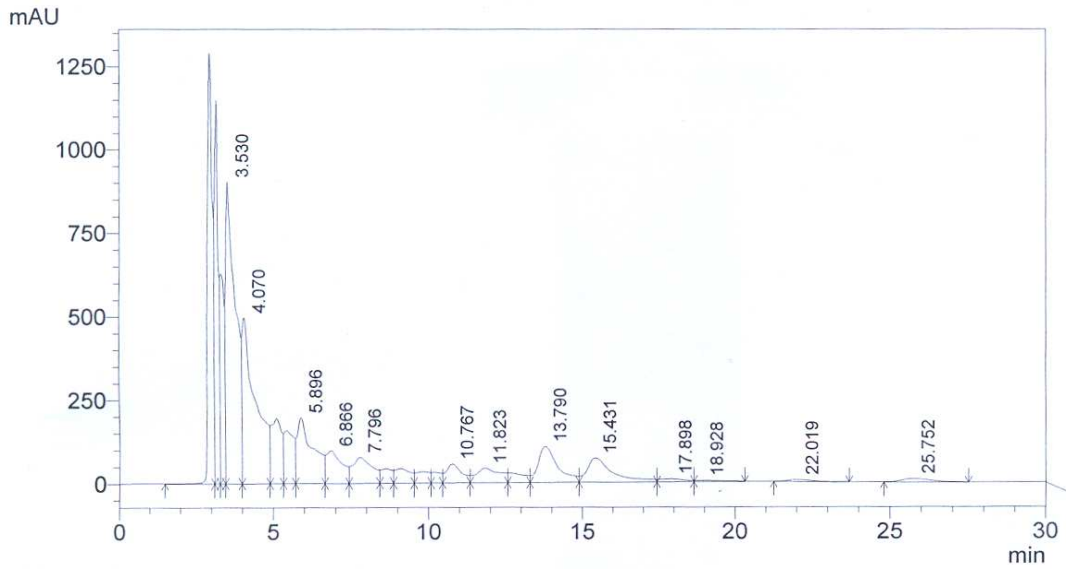


**Figure 1.** HPLC spectrum of leaf hot aqueous extract of *Ximenia americana* (XA).

The HPLC spectrum showed thirteen peaks with retention times in minutes of 3.530, 4.070, 5.896, 6.886, 7.796, 10.767, 11.823, 13.790, 15.431, 17.898, 18.928, 22.019 and 25.752. Compounds with retention time in minute of 6.886 and 7.796 minute corresponded to rutin and ferullic acid respectively.

### Growth inhibitory effects of XA on *Sorghum bicolor* seed

There was an appreciable reduction on the length of radicles of *Sorghum bicolor* seeds treated with the various concentration of the extract. The seed radicle lengths increased with the incubation period of 48-96 h. A rapid and progressive growth was observed in the water control seeds radicle lengths. At 48 h, percentage seed

growth inhibition was 28.43% for seeds treated with 1 mg/ml of HC. Then at 96 h, the mean radicle lengths of the control seeds was 69.50±4.06 mm while the mean radicle length of the seeds treated with 1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml 16 mg/ml, 24 mg/ml, 32 mg/ml, 40 mg/ml and 48 mg/ml were 53.35±1.34 mm, 49.30±2.48 mm, 48.18±1.21 mm, 42.90±0.84 mm, 35.05±3.53 mm, 34.48±3.20 mm, 24.60±0.38 mm, 14.13±1.23 mm, and 12.50±1.32 mm as shown in Fig. 2, corresponding to percentage inhibitions of 23.24%, 29.06%, 30.68%, 38.27%, 49.57%, 50.39%, 64.60%, 79.67% and 82.01%, respectively.

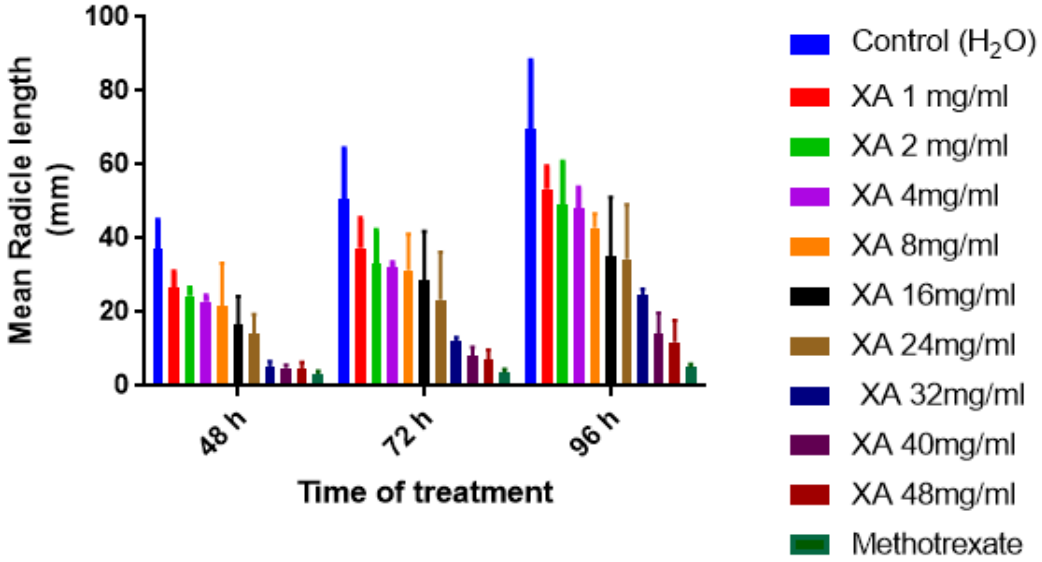


**Figure 2.** Inhibitory effects of *Ximenia americana leaf* aqueous extract (XA) on the growth of *Sorghum bicolor* seed radical.

**Table 1.** Mean radical length, percentage inhibition and percentage growth for *Sorghum bicolor* seeds treated with XA.

| Treatment | Mean radicle length | | | % Inhibition* | | | % Growth† | | |
|---|---|---|---|---|---|---|---|---|---|
| | 48 h. | 72 h. | 96 h. | 48 h. | 72 h. | 96 h. | 48 h. | 72 h. | 96 h. |
| Control ($H_2O$) | 37.28±1.66 | 50.48±3.00 | 69.5±4.06 | 0 | 0 | 0 | 100 | 100 | 100 |
| methotrexate | 3.28±0.17 | 3.73±0.18 | 5.03±0.21 | 91.20 | 92.61 | 92.76 | 8.80 | 7.39 | 7.24 |
| XA (1 mg/ml) | 26.68±0.92 | 37.38±1.75 | 53.35±1.34 | 28.43 | 25.95 | 23.24 | 71.57 | 74.05 | 76.76 |
| XA (2 mg/ml) | 24.38±0.48 | 33.03±1.98 | 49.30±2.48 | 34.60 | 34.57 | 29.06 | 65.40 | 65.43 | 70.94 |
| XA (4 mg/ml) | 22.90±0.34 | 32.00±0.31 | 48.18±1.21 | 38.57 | 36.61 | 30.68 | 61.43 | 63.39 | 69.32 |
| XA (8 mg/ml) | 21.58±2.55 | 31.45±2.13 | 42.90±0.84 | 42.11 | 37.70 | 38.27 | 57.89 | 62.30 | 61.73 |
| XA (16 mg/ml) | 16.53±1.70 | 28.00±2.86 | 35.05±3.53 | 55.66 | 44.53 | 49.57 | 44.34 | 55.47 | 50.43 |
| XA (24 mg/ml) | 14.03±1.18 | 23.35±2.82 | 34.48±3.20 | 62.37 | 53.74 | 50.39 | 37.63 | 46.26 | 49.61 |
| XA (32 mg/ml) | 5.03±0.36 | 12.25±0.22 | 24.60±0.38 | 86.51 | 75.73 | 64.60 | 13.49 | 24.27 | 35.40 |
| XA (40 mg/ml) | 4.88±0.17 | 8.30±0.49 | 14.13±1.23 | 86.91 | 83.56 | 79.67 | 13.09 | 16.44 | 20.33 |
| XA (48 mg/ml) | 4.95±0.40 | 7.25±0.57 | 12.50±1.32 | 86.72 | 85.64 | 82.01 | 13.73 | 14.36 | 17.99 |

*Percentage Inhibition = [(mean radicle length of control - mean radicle length of treated) / mean radicle length of control] X 100. †Percentage growth = 100 - percentage inhibition, n = 20. $p<0.0001$.

Therefore, the inhibitory effect of XA was concentration-dependent. Inhibitory effects of *Ximenia americana* aqueous extract on the growth of *Sorghum bicolor* seed radicle was determined for different concentrations: 1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml, 16 mg/ml, 24 mg/ml, 32 mg/ml, 40 mg/ml and 48 mg/ml. Radicle lengths (mm) were measured at 48, 72 and 96 h. Distilled water without *Ximenia americana extract* was used as negative control, methotrexate 0.167 mg/ml was used as positive control. Mean radicle length ± standard error of mean, percentage growth inhibition and percentage growth at 48, 72 and 92 h for seeds treated with 1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml, 16 mg/ml, 24 mg/ml, 32 mg/ml, 40 mg/ml, 48 mg/ml aqueous extract of *X. americana* as well as the negative control, and methotrexate 0.167 mg/ml used as positive control are as shown in Table 1.

## DISCUSSION

As a preliminary and preparatory assay to antiproliferative test on a cancer cell line system, the radicle lengths of fast growing seeds such as *Sorghum bicolor* have been utilized as a parameter for the testing of suspected anticancer agents. Generally, cancer cells have a characteristic of fast proliferation, and this is also the case with meristematic cells of *S. bicolor* seeds when exposed to favourable conditions [18]. Hence, the use of the method for this study.

The phytochemical analysis of XA showed the presence of tannins, saponins, flavonoids, and carbohydrates. The HPLC spectrum of XA revealed the presence of rutin and ferulic acid at retention times of 6.886 and 7.796 minutes, respectively (Fig. 1). Flavonoids have been reported to have relatively low toxicity compared to other metabolites like alkaloids. Flavonoids have also been referred to as 'natural biological response modifiers' because of the strong experimental evidence of their ability to modify the body's reactions to allergens, viruses and carcinogens. Flavonoids have been reported to possess anti-allergic, anti-inflammatory, antimicrobial and anticancer activities. Saponins have been reported to exhibit antioxidant, anticancer and anti-inflammatory activities and tannins to have antibacterial, antiviral and anti-tumor activities. Generally, the pharmacological properties of medicinal plants depend on their secondary metabolites constitution [19].

*Ximenia americana* had been reported to possess anticancer properties. Physicochemical characterization showed that the active antineoplastic components of the plant material were proteins with galactose affinity [20]. Cytotoxic and antiproliferative activities of *Ximenia americana* against six cancerous cells lines had been reported. The study revealed the presence of flavonoids (13%), gallotannins (5%), phenolic acids (0.7%), ellagic acid (0.3%) and an abundance of condensed tannins (81%) [21]. This finding is in agreement with the results obtained with the Nigerian material (Fig. 1) reported herein, where rutin and ferullic acid were identified as the bioactive constituents of *Ximenia americana* leaf.

In a review of the chemistry, pharmacology and biological properties of *Ximenia americana*, the chemical constituents and anticancer properties were reported [22]. Antioxidant activity study and total phenolic determination of leaf extracts of *Ximenia americana*, an anti-tumor plant used traditionally in Mali, revealed the presence of phenolic compounds with potential anticancer properties [23]. Flavonoids have been reported to possess anticancer potential [24]. Rutin isolated from *Triticum aestivum* showed anticancer activity [25]. Currently it has been observed that cinnamic acid and its analogs such as ferulic acid and isoferulic acid display various pharmacological activities including anticancer [26].

## CONCLUSION

The hot aqueous extract of *Ximenia americana* leaf (XA) exhibited growth inhibitory effects on fast proliferating cells of *S. bicolor* seed radicle. Hence, by extension it can inhibit cancerous cells. This study provided preliminary evidence that supports the ethno medicinal use of *X. americana* leaf growing in Nigeria for the treatment of breast cancer. The observed growth inhibitory properties may be attributable to the flavonoid rutin and ferulic acid content.

## AUTHORS' CONTRIBUTION

ACN wrote the initial draft of the manuscript; SEO designed the study and did the statistical analysis; UEB proof read, and edited the word. All authors were involved in the execution of the research plan. The final manuscript was read and approved by all authors

## TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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