GVU-J.SHT *GVU Journal of Science, Health and Technology*

GVU-J. SHT 2025,**10**; **1**, 161–164, doi:10.5281/zenodo.16461368

Studies on the Toxicity and Anticancer Properties of Stem-Bark Extract of *Prosopis africana* Plant

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Received: *25 May 2025 []* ***Published:*** *18 June 2025*

This study investigated the anticancer and hepatoprotective potential of *Prosopis africana* stem bark fractions in 7,12-dimethylbenz[α]anthracene (DMBA)-induced mammary cancer in female albino Wistar rats. The stem bark was extracted using ethanol, and fractions (F1, F2, F3) were obtained via column chromatography. Acute toxicity testing revealed dose-dependent mortality at an LD_{50} of 5000 mg/kg, indicating low toxicity. DMBA induction significantly elevated cancer antigen 125 (CA125) levels (48.52 IU/L vs. normal control: 14.48 IU/L), confirming tumor development. Treatment with *Prosopis africana* fractions, particularly F1 at 500 mg/kg, markedly reduced CA125 levels (32.49 IU/L, 33% decrease), demonstrating potent anticancer activity. Additionally, DMBA-induced liver damage, evidenced by elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, was significantly mitigated by F1 (ALT: 30.66 IU/L; AST: 38.32 IU/L), suggesting hepatoprotective effects. Fraction F1 exhibited superior efficacy compared to F2 and F3. These findings highlight *Prosopis africana*'s therapeutic potential as a natural anticancer and hepatoprotective agent, supporting its use in traditional medicine.

Keywords: Anticancer, breast, mortality, *Prosopis africana*, toxicity

Introduction

Medicinal plants and human beings have had a unique relationship since time immemorial. Man's vital interest in plants, primarily as a source of food, shelter, and clothing, dates back to the very origin of human civilization (Idris and Idris, 2019). Tribal people have traditionally used medicinal plants as traditional treatments for various illnesses, such as rheumatism, diabetes, inflammation, stomach problems, and even insect poisoning. Because of their distinct chemical characteristics, various bioactive substances, including phenolic compounds, flavonoids, terpenoids, saponins, and essential oils, have been extracted from these plants and have shown protective effects (Manjamalai et al., 2012).

Prosopis africana (Guill. & Perr.) Taub (Mimosaceae) is a plant commonly found in sub-Saharan Africa, and it has been used in traditional medicine for centuries due to its various therapeutic properties (Ajiboye et al., 2013; Yanda et al., 2022). The stem bark of this plant has been reported to contain several phytochemicals, such as alkaloids, flavonoids, tannins, and saponins, which have been linked to its anti-inflammatory, analgesic, antidiabetic, and antimicrobial activities (Yanda et al., 2022). Despite its widespread use in traditional medicine, there is a lack of adequate scientific data on the toxicity profile of *Prosopis africana* (Parasuman, 2011).

Cancer is a complex and multifaceted disease characterized by the uncontrolled growth and spread of abnormal cells. It arises due to genetic mutations that disrupt normal cellular functions, leading to uncontrolled proliferation, evasion of cell death, and metastasis (Weimberg and Hanaham, 2021). Cancer is a leading cause of death worldwide, with an estimated 10 million deaths in 2020 alone (Sung et al., 2021). The disease manifests in various forms, including carcinomas, sarcomas, leukemias, lymphomas, and central nervous system cancers, each with distinct biological behaviors and treatment challenges (Vogalstein and Kinzlar, 2022).

Medically, breast cancer is considered the most frequent malignant tumor that begins in the breast cells and can spread to other parts of the body. Breast cancer represents a global health burden, and its incidence continues to rise, accounting for a significant proportion of mortality in women (Jemal et al., 2021). In the United States, the number of new breast cancer cases and deaths has been estimated at 268,600 and 41,760, respectively (Clement et al., 2020). Although the incidence in Black women is lower than in White women, the mortality rate is higher, possibly due to differences in diet and lifestyle (DeSantis et al., 2019). In Nigeria, breast cancer incidence has surpassed that of cervical cancer. This may be attributed to factors such as delayed first childbirth, shorter duration of breastfeeding, family history of breast cancer, and late menopause (Morounke et al., 2017).

7,12-Dimethylbenz[α]anthracene (DMBA) is a procarcinogen commonly used to induce breast cancer in experimental rats.

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It undergoes metabolic activation to form the carcinogenic compound dihydrodiol epoxide. This process involves mixed-function oxidases located in rat liver microsomes. The resulting metabolite binds with adenine residues of deoxyribonucleic acid, leading to mutagenesis and carcinogenesis (Jayakumar et al., 2016).

The aim of this study is to investigate the therapeutic properties of *Prosopis africana* stem bark extract and assess its potential use as a natural anticancer agent.

Materials and Methods

Sample Collection, Preparation, and Extraction

The stem bark of *Prosopis africana* was collected from Girei Local Government Area of Adamawa State, Nigeria, and dried under shade at room temperature in the Chemistry Department, Modibbo Adama University, Yola, Adamawa State. The sample was ground to powder using a mortar and pestle. Soxhlet extraction was employed to obtain the crude extract of the stem bark using ethanol as the solvent.

Chromatographic Analysis of Prosopis africana Ethanolic Extract

Column chromatography and thin-layer chromatography were used to separate and identify compounds in the ethanolic extract of *Prosopis africana*. These chromatographic techniques facilitated the isolation of various fractions, which were analyzed for their constituent compounds. Column chromatography was carried out using ethyl acetate and n-hexane as the mobile phase. After preparing the column, the stationary phase was packed into the column, the sample was loaded, and elution was performed with the mobile phase. Compounds were separated based on polarity, and the fractions were collected as the mobile phase eluted from the column (Bilyaminu et al., 2023).

Preparation of 7,12-Dimethylbenz[α]anthracene (DMBA)

Exactly 20 g of DMBA was dissolved in 100 mL of water (Parasuraman, 2011).

Animal Collection

Female albino rats weighing 120 g ± 20 g were obtained from the National Veterinary Research Institute (NVRI), Plateau State, Nigeria. The rats were housed in propylene cages and given a standard grower diet (Vital Feeds) and water. They were maintained under laboratory conditions of 28°C and a 12-hour light/dark cycle for seven days to allow acclimatization before the commencement of the experiment (Obode et al., 2020).

Sub-acute Toxicity Test

The acute toxicity test was conducted following the method described by Lorke (1983) and adopted by Karber (2007) with slight modifications. The test involved administering doses according to body weight. Twenty albino rats (120 g ± 20 g) were grouped into five groups of four rats each. The animals received graded doses ranging from 1000 mg/kg to 5000 mg/kg. The rats were observed for 24 hours for behavioral changes and mortality. The median lethal dose (LD_{50}) was calculated using Karber's arithmetical method as follows:

$$LD_{50} = LD_{100} - \sum \left(\frac{a \times b}{n} \right)$$

where LD_{50} = median lethal dose, LD_{100} = least dose required to kill 100%, a = dose difference, b = mean mortality, and n = group population (Bilyaminu et al., 2023).

Experimental Design of the Cancer Test

Forty rats were randomly divided into eight groups of five rats each. Group 1 served as the normal control (no DMBA induction). Breast cancer was induced in Groups 2 to 8 using DMBA at a dose of 15 mg/kg body weight. Group 2 served as the experimental control. After four weeks of DMBA induction, rats were treated with *Prosopis africana* fractions (F1, F2, and F3) at doses of 250 mg/kg and 500 mg/kg body weight, administered consecutively for four weeks (Hussaini et al., 2023).

Histological Investigation

Mammary gland biopsies were excised from the experimental animals of each group after blood sample collection. The tissues were fixed in 10% neutral formalin solution, processed in an alcohol-xylene series, embedded in paraffin, and sectioned at 0.45 microns using a microtome. The sections were stained with hematoxylin and eosin and examined microscopically for histopathological evaluation (Akshatha, 2018).

Statistical Analysis

Results were expressed as mean ± standard error of the mean (SEM). One-way ANOVA was used to compare means among groups.

Results and Discussion

Acute Toxicity Test of Prosopis africana Using Albino Wistar Rats

The acute toxicity test results of *Prosopis africana* stem bark extract are presented in Table 1. No deaths were observed in Groups 1 to 3, which received doses of 1000–3000 mg/kg. One death occurred in Group 4 (4000 mg/kg), and two deaths occurred in Group 5 (5000 mg/kg). All animals survived phase one of the toxicity test. Only the animals administered with the highest doses (4000 and 5000 mg/kg) died. The LD_{50} of the extract was calculated as 4812.5 mg/kg. A similar study by Abubakar et al. (2023) using a methanolic extract of the stem bark reported an LD_{50} of 3807.88 mg/kg.

Table 1 Acute Toxicity Test of *Prosopis africana* Stem Bark Extract

Group	Dose (mg/kg)	Dose Diff. (a)	Dead	Mean Dead (b)	Product (a × b)
1	1000	1000	0	0	0
2	2000	1000	0	0	0
3	3000	1000	0	0	0
4	4000	1000	1	0.25	250
5	5000	1000	2	0.50	500

Column Chromatographic Analysis of Prosopis africana Stem Bark Crude Extract

The results of the column chromatography analysis are shown in Table 2. Fraction 1 had the highest yield (32.4%) and was dark brown in color. Fractions 2 and 3 were brown and yellow, with yields of 20.8% and 12.4%, respectively. These results may assist in identifying specific compounds, optimizing chromatographic conditions, and guiding future scale-up processes (Bilyaminu et al., 2023).

Table 2 Column Chromatographic Analysis of *Prosopis africana* Stem Bark Extract

Fraction	Mass (g)	Percentage Yield (%)	Color
1	8.1	32.4	Dark brown
2	5.2	20.8	Brown
3	3.1	12.4	Yellow

Anticancer Investigation of the Fractions of Prosopis africana Stem Bark

Table 3 presents the effects of *Prosopis africana* fractions on cancer antigen 125 (CA125) and the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST). CA125 is a tumor marker commonly elevated in cancer conditions. The normal control group had a mean CA125 level of 14.480 ± 0.679 IU/L, while the negative control group showed a significantly elevated level (48.523 ± 1.418 IU/L), confirming successful cancer induction.

Treatment with fractions (F1, F2, F3) led to dose-dependent reductions in CA125 levels, with F1 at 500 mg/kg showing the most notable decrease (32.487 ± 0.857 IU/L). Similarly, ALT and AST levels were highest in the negative control group (43.453 ± 2.283 IU/L and 50.026 ± 0.549 IU/L, respectively), but decreased significantly in the treatment groups, particularly at the 500 mg/kg dose of F1 (ALT: 30.660 ± 0.308 IU/L; AST: 38.320 ± 1.211 IU/L).

These reductions suggest both anticancer and hepatoprotective effects of the fractions. The data indicate F1 was the most effective fraction across all tested parameters. However, further studies are needed to confirm these findings and elucidate the underlying mechanisms (Hussaini et al., 2012).

Conclusion

This study investigated the therapeutic potential of *Prosopis africana* stem bark fractions (F1, F2, and F3) in DMBA-induced mammary cancer in rats. Acute toxicity testing revealed no mortality at doses ≤ 3000 mg/kg, and the calculated LD_{50} exceeded 5000 mg/kg, indicating low toxicity.

Table 3 Anticancer Activity of *Prosopis africana* Fractions on CA125, ALT, and AST Levels

Group	CA125 (IU/L)	ALT (IU/L)	AST (IU/L)
Normal control	14.480 ± 0.679	21.183 ± 0.580	27.646 ± 1.191
Negative control	48.523 ± 1.418	43.453 ± 2.283	50.026 ± 0.549
F1 250 mg/kg	39.286 ± 0.448	34.910 ± 0.998	42.183 ± 0.455
F1 500 mg/kg	32.487 ± 0.857	30.660 ± 0.308	38.320 ± 1.211
F2 250 mg/kg	40.863 ± 0.759	39.453 ± 0.355	42.010 ± 0.929
F2 500 mg/kg	39.416 ± 0.591	35.310 ± 0.927	40.150 ± 0.364
F3 250 mg/kg	40.830 ± 0.601	39.816 ± 0.268	40.776 ± 1.345
F3 500 mg/kg	40.190 ± 0.383	37.796 ± 0.606	37.883 ± 0.627

In the anticancer activity assessment, DMBA induction significantly elevated CA125 levels (48.52 IU/L) compared to normal values (14.48 IU/L). Treatment with Fraction F1 (500 mg/kg) produced the most substantial reduction in CA125 levels (32.49 IU/L), corresponding to a 33% decrease. Fractions F2 and F3 showed moderate reductions (15–18%).

Regarding hepatoprotection, DMBA exposure elevated ALT and AST levels (43.45 IU/L and 50.03 IU/L, respectively), indicating liver injury. Fraction F1 (500 mg/kg) restored ALT and AST levels to 30.66 IU/L and 38.32 IU/L, respectively, approaching normal values.

Overall, *Prosopis africana* extract demonstrated safety at moderate doses (LD_{50} > 5000 mg/kg). Among the tested fractions, F1 (500 mg/kg) exhibited the most effective antitumor and hepatoprotective effects, supporting its traditional medicinal use and suggesting its potential for further therapeutic development.

Conflict of Interest

The authors declare no competing interest.

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