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Effect of Methanol Stem Bark Extract of *Annona senegalensis* as an Antibacterial Agent and on Liver Enzyme Markers in Diethyl Nitrosamine-Induced Hepatocellular Carcinoma in Rats

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Abstract

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related deaths worldwide, frequently linked to exposure to chemical carcinogens such as diethylnitrosamine (DEN). Medicinal plants have emerged as promising sources of bioactive compounds with potential anticancer and antimicrobial properties. This study evaluated the therapeutic potential of methanol stem-bark extract of *Annona senegalensis* against DEN-induced hepatocellular carcinoma and selected bacterial pathogens. Fifty-four male albino rats were allocated into six groups, with hepatocarcinogenesis induced by DEN, followed by a 14-day treatment with varying doses of the extract. Hepatoprotective effects were assessed using liver enzyme markers and serum protein levels, while antibacterial activity was determined via disc diffusion

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assays against *Staphylococcus aureus* and *Escherichia coli*. Results demonstrated that the extract significantly reduced liver enzyme levels (ALT, AST, ALP) and improved serum proteins (total protein and albumin) at higher doses (400 and 600 mg/kg), indicating hepatoprotective potential. Bilirubin levels also improved, suggesting reduced liver stress. Antibacterial evaluation revealed dose-dependent activity, with strong inhibition against *S. aureus* comparable to tetracycline at 200 µg/mL, and moderate efficacy against *E. coli*. These findings highlight the dual therapeutic potential of *A. senegalensis* stem-bark extract as both a hepatoprotective and antibacterial agent. Future studies are recommended to isolate and characterize the bioactive compounds responsible for these effects and to expand antibacterial evaluation to a wider spectrum of pathogens.

Keywords: Stem-Bark Extract; *Annona senegalensis*; Antibacterial Activity; Liver Enzyme Markers; Diethylnitrosamine; Hepatocellular Carcinoma; Wistar Rats

INTRODUCTION

Medicinal plants, also known as medicinal herbs or herbal remedies, are plants that have been historically and culturally used for their therapeutic properties (Jamshidi-Kia et al., 2017). These plants contain various compounds that can have beneficial effects on the body and are often used as alternatives or complements to conventional medicine. A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Alamgir, 2017). Medicinal plants refer to plants that are used for their therapeutic properties and have been traditionally employed in the treatment and prevention of various ailments (Sofowora et al., 2013). These plants contain active chemical compounds that have pharmacological effects on the human body. Many civilizations throughout history have recognized and utilized the healing potential of medicinal plants (Jamshidi-Kia et al., 2017).

Numerous plants have been studied for their medicinal properties and have been incorporated into different traditional systems of medicine, such as Traditional Chinese Medicine, Ayurveda, and Indigenous healing practices Numerous plants have been studied for their medicinal properties and have been incorporated into different traditional systems of medicine, such as Traditional Chinese Medicine, Ayurveda, and Indigenous healing practices Medicinal plants can be found in various forms, including leaves, flowers, roots, seeds, bark, or even the whole plant (Van Wyk and Wink, 2018). They can be consumed

orally, applied topically, or used in the form of extracts, tinctures, teas, or poultices. Numerous plants have been studied for their medicinal properties and have been incorporated into different traditional systems of medicine, such as Traditional Chinese Medicine, Ayurveda, and Indigenous healing practices (Jaiswal et al., 2016).

Some well-known medicinal plants include aloe vera, garlic, echinacea, ginger, peppermint, chamomile, ginseng, milk thistle and St. John's wort. Aloe vera is known for its soothing and healing properties, often used for skin conditions and minor burns (Maan et al., 2018). Garlic is believed to have antibacterial, antiviral, and antifungal properties. Echinacea is used to boost the immune system and treat colds and infections. Ginger contains the compound curcumin, which has anti-inflammatory and antioxidant effects (Ballester et al., 2022). Peppermint is often used to alleviate digestive issues such as indigestion and nausea. Chamomile is used for its calming and relaxing properties, often consumed as a tea for sleep and stress relief. Ginseng is known for its adaptogenic properties and used to promote vitality and stamina (Irfan et al., 2020).

Medicinal plants contain abundant bioactive compounds, typically produced as a defense mechanism against invaders. These bioactive compounds are referred to as phytochemicals, which are secondary metabolites (Akbar et al., 2024). Saponins have ability to lower cholesterol. Tannins which are natural antibiotics. phenols and flavonoids which are rich in antioxidants. Alkaloids which are rich in nitrogenous compounds and stimulant. Cardiac glycosides which are good cardiovascular drugs.Hepatocellular carcinoma (HCC) is one of the most prevalent forms of liver cancer worldwide, accounting for a significant proportion of cancer-related deaths (Sayiner et al., 2019). It is often associated with underlying liver diseases, such as hepatitis infections, cirrhosis, and exposure to carcinogenic compounds like diethyl nitrosamine. Diethyl nitrosamine (DEN) is a type of nitrosamine, a known hepatocarcinogen forming DNA adducts in the liver (Tolba et al., 2015). The induction of oxidative stress by DEN further contributes to the pathogenesis of hepatocellular carcinoma. DEN and other known hepatocarcinogens induce hepatic injury by enhancing the proliferation of cells, which is accompanied by hepatocellular necrosis (You et al., 2021; Tolba et al., 2015). They target the liver where they are bio-transformed via the cytochrome p450-dependent mechanism, CYP2E1, explicitly resulting in DNA-adducts (Tolba et al., 2015). Hepatic carcinogenesis is ranked the fifth most prevalent cancer and is usually influenced by agents such as alcohol, phenobarbital, 2-acetylaminofuorene, and DEN (Unsal and Belge-Kurutas, 2017).

Animal models are viewed as a critical tool for studying hepatic carcinogenesis and are often used for cancer research. Studies have reported early induction of oxidative stress, inflammation, and proliferation by DEN in rat models (Mansour et al., 2019). When injected into young mice, DEN targets the liver, where it is biologically transformed into alkylating agents that can create mutagenic DNA adducts by centrilobular hepatocytes (Connor et al., 2018). Additionally, there is proof that inflammation plays a role in the development of hepatocarcinogenesis brought on by DEN (Xu et al., 2021). In addition to being a genotoxic substance, DEN is also hepatotoxic and causes necrotic cell death. This damage sets off an inflammatory response that increases the expression of mitogens such as interleukin-6 (IL-6), which encourages the proliferation of remaining hepatocytes as a form of reparative action (Hou et al., 2018).

Medicinal plants and herbs have been a cornerstone of traditional medicine for centuries and have gained significant attention in modern pharmacology due to their bioactive compounds (Izah et al., 2024). They contain phytochemicals and natural sources of antioxidants. Annona senegalese (AS) is one such plant containing an abundance of phytochemicals; it contains alkaloids, flavonoids, glycosides, tannins, saponins, steroids, and anthocyanins (Johnson et al., 2017; Musa et al., 2017). These bioactive compounds have been reported to exhibit anticancer activities by inducing apoptosis, inhibiting angiogenesis, and modulating oxidative stress pathways. AS is often referred to as wild soursop; the plant is a 25m tall shrub having blue to greenish, oblong, alternate, and simple leaves. It is prominent among the Northern region of Nigeria and commonly called in Hausa language, Gwandar dajì (Okhale et al., 2016).

Almost all the plant parts have been reported useful for medicinal applications. The leaves are used for yellow fever, tuberculosis, and smallpox treatment while the stem bark is employed for treating hernia and snakebite (Djoza et al., 2017; Al Kazman et al.,2022). The root is employed to ease swallowing, impotence, and an antidote for poison; root bark helps in the treatment of infectious diseases while the juice from the plant dries up chickenpox on the skin (Emmanuel and Mamoudou, 2015). Several studies have documented its biological activities such anticonvulsant, antimicrobial, antiplasmodial, anti-venomous, spermatogenic, anti-inflammatory, analgesic and antihelmintic activities (Emmanuel et al., 2014; Jada et al. 2015).

Statement of Problem

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related deaths globally, with its incidence rising due to exposure to environmental carcinogens, chronic viral hepatitis infections, and lifestyle-related liver diseases (Yang et al., 2019). Conventional treatment options such as chemotherapy and radiotherapy often result in severe side effects, high costs, and limited long-term survival benefits, highlighting the urgent need for alternative therapies that are effective and less toxic. Diethylnitrosamine is a well-established hepatotoxin and carcinogen that induces liver cancer through oxidative stress, DNA damage, and chronic inflammation (Mansour et al., 2019). However, plant-based remedies that can counteract diethylnitrosamine-induced liver damage remain underexplored, with limited research on natural products capable of modulating liver enzyme markers to provide evidence of hepatoprotection and anticancer efficacy.

The increasing prevalence of antibiotic-resistant bacteria represents a significant global health threat, necessitating the discovery of new antimicrobial agents. Annona senegalese is known for their richness in bioactive compounds and shows promise in addressing this issue, yet the antibacterial efficacy of its methanol leaves extract has not been adequately investigated. The dual challenge of combating liver cancer and bacterial infections emphasizes the need for a comprehensive study into the therapeutic potential of A. senegalese.

Justification of the Problem

This study seeks to evaluate the therapeutic potential of Annona senegalese methanol stem-bark extract, in addressing a particular type of liver cancer (hepatocellular carcinoma). The use of diethylnitrosamine to induce hepatocarcinogenesis in experimental models provides a reliable framework to study the effects of potential therapeutic agents, while liver enzyme markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) offer critical indicators of liver function and damage. Investigating the ability of A. senegalese to modulate these markers could provide scientific evidence of its hepatoprotective and anticancer potential.

The antibacterial properties of A. senegalese methanol stem-bark extract are significant in light of the growing global health threat posed by antibiotic-resistant bacteria. Exploring its antibacterial activity against pathogenic microorganisms offers the potential for a natural alternative to synthetic antibiotics, which are increasingly losing efficacy due to

resistance. The dual therapeutic potential of A. senegalese justifies this research, as it seeks to tackle two critical and interconnected health challenges (cancer and bacterial infections) with a single natural solution. This comprehensive approach could improve public health outcomes by offering cost-effective, accessible, and safer therapeutic options.

Aim and Objectives

The aim of this study is to evaluate the effect of methanol stem-bark extract of Annona Senegalese as an antibacterial and on liver enzyme markers on diethyl nitrosamine-induced hepatocellular carcinoma in rats.

The objectives include:

i. Evaluation of the effect of the methanol stem-bark extract of Annona senegalese on Cancer-Induced diethyl nitrosamine in rats.
ii. Estimation of the level of liver enzyme markers.
iii. Evaluation of antibacterial potentials of the methanol stem-bark extract of Annona senegalese.

Annona Senegalese

Annona senegalese, commonly referred to as African custard apple, wild custard apple, wild soursop, abo ibobo (in Yoruba), sunkungo (in Mandinka), and dorgot (in Wolof), belongs to the Annonaceae family. The species name senegalese signifies its origin from Senegal, where the type specimen was first collected (Romeiras et al., 2018). This plant serves as a traditional food source in Africa, with its fruits offering potential benefits such as enhanced nutrition, improved food security, rural development, and sustainable land management.

A. senegalese grows as either a shrub or a small tree, typically reaching heights between 2 and 6 meters, though it can occasionally grow up to 11 meters. Its bark varies from smooth to rough in texture and has a gray-silver or gray-brown hue, often marked with leaf scars and peeling in rounded flakes that reveal lighter underbark. Young branches are covered in thick, gray, brown, or yellowish hairs, which diminish with age (AgroForestry Tree Database, 2013). The leaves are green to blue-green, alternate, simple, and range from oblong to ovate or elliptic in shape, measuring between 6–18.5 cm in length and 2.5–11.5 cm in width. The upper surface of the leaves is mostly hairless, while the underside may be hairy, often displaying reddish aracnose veins. The leaf apex is either

rounded or slightly notched, with a squared or barely lobeliar base and an entire margin. The petioles are stout, measuring between 0.5–2.5 cm in length.

The flowers of A. senegalese grow singly or in clusters of two to four from the leaf axils, reaching up to 3 cm in diameter on 2 cm stalks (Okhale et al., 2016). Each flower has six thick petals arranged in two whorls, green on the exterior but either creamy or reddish inside, measuring around 0.8–1.5 cm in length and 0.9–1.1 cm in width. They may be smooth or slightly fuzzy. The inner whorl of petals curves over the stamens and ovary, while the three smaller sepals are oval in shape, measuring about 3–4 mm by 4–5 mm. The stamens vary in length from 1.7 to 2.5 mm. The flowering period typically occurs between April and June, with pollen released in tetrads. The plant produces compound fruits consisting of numerous fused, fleshy, and bumpy carpels, which are oval or round, measuring approximately 2.5–5 cm in length and 2.5–4 cm in width. Initially green, the fruit ripens to yellow and eventually turns orange, containing multiple oblong, cylindrical seeds of a burnt-orange color. The fruit stalk measures between 1.5–5 cm in length. A. senegalese is predominantly pollinated by beetles but can also be manually pollinated when cultivated as a crop. Its seeds have a viability period of about six months (AgroForestry Tree Database, 2013).



Figure 1: Annona senegalese

Uses of Annona senegalese

The primary use of this versatile plant is for food, but it has applications in numerous aspects of human endeavor, and every part of the plant has unique properties and uses. The flowers, leaves and fruit are edible and culinary: white fruit pulp has a mild, pineapple-like flavor (AgroForestry Tree Database, 2013). Flowers are added to spice or garnish meals; leaves are eaten by humans as vegetables, or grazed by livestock. Leaves are also part of the diet of the West African giraffe. The leaves are also used to create a general health tonic, in the treatment of pneumonia, and as mattress and pillow stuffing. Specific to Sudan, leaves are boiled in the making of perfume. Bark can be processed to produce yellow-brown dye, insecticide, or medicine for treating a wide array of ailments, including worms parasitic on the intestines or flesh (notably guinea worms), diarrhea, gastroenteritis, lung infections, toothaches, and even snakebites. Natural gum in the bark is used to close open wounds (AgroForestry Tree Database, 2013).

Roots are also used medicinally in treating a gamut of conditions, from dizziness and indigestion to chest colds to venereal diseases. Suckering shoots provide binding fibers, and the malleable, pale brown to white wood is used to carve tool handles, or fashioned into poles. Wood ash is an admixture to chewing tobacco and snuff, and also in soap production as solvent. The essential oils in the fruits and leaves are valued for their organic chemical constituents: car-3-ene (in fruit) and linalool (from leaves). Certain parts of A. senegalese are used in treating skin or eye disorders. Many South Africans believe the roots can cure insanity. Some Mozambicans feed them to infants to wean them from their mother's breast (AgroForestry Tree Database, 2013).

MATERIALS AND METHODS

Materials

Apparatus

Electric blender (9X1000 Newclime France), Micropipette (CE-IVD Lambmat India), spectrophotometer (UV 751 Shangahi Youke Instrument Co LtD China), Water Bath (HH-W21-Cr42II India Mart India), Weighing Balance (PB 3002-5 Mettler Toledo Switzerland), DYMIND Haematological analyser (DYMIND DH76 Biotech, China).

Reagents

Diethyl nitrosamine (DEN)-Induced Hepatocellular Carcinoma, Silymarin Standard, EDTA Bottles and Ethanol.

Plant Material

The stem-bark extract of *Annona senegalese* was obtained from the vicinity of the Federal University of Wukari.

Methods

Preparation of Plant Extract

The collected plant materials were washed sliced and completely shade dry. The dried material was ground make to a fine powder and used for extraction. The powdered plant material *Annona senegalese* (200 g) was extracted with methanol (1 litre) in an air tight clean flat-bottomed container for 48 hours at room temperature with occasional stirring and shaking (Trease and Evans, 2002). The methanol extract was filtered first through a fresh cotton plug and then through a whatman filters. The filtrate was evaporated to dryness in vacuo by a rotary evaporator at 40-50°C and the extract was kept in a well tight sterile bottle/container under refrigerated conditions until use.

Animals

Fifty-four (54) male albino rats (weighing between 140 ± 20 g) were obtained from HAUEMM Veterinary Animal House, Federal Housing Estate. Adamawa state, Nigeria. They were housed in polypropylene cages, and were given standard grower diet (Vital Feeds, Jos) and water ad labium for 7 days to enable them acclimatize before the commencement of the experiment. Throughout the experiment it was maintained under the laboratory conditions of 29 ± 2°C (temperature) and 12 hours light and dark cycle in the Department of Biochemistry, Federal University Wukari, Taraba, Nigeria. Guide for the Care and Use of Laboratory Animals was strictly followed.

Experimental Design

The rats will be randomly divided into six equal groups of six rats each. Group I served as normal control, that is, no inducement and no administration of methanol extract as shown in the group treatment below. Liver carcinogenesis was induced in group II, III, IV V, and VI, by injecting diethylnitrosamine (in DMSO) intraperitonially at a dose of 50 mg/kg body weight once in a week for a period of three weeks as reported by Memon et

al., *et al.*, (2020). Group II served as the negative control while group III served as the positive control group (silymarin 100 mg/kg b.w. was used as standard drug). The methanol extract of the plant extract was administered to group IV (200 mg/kg b.w.) and group V (400 mg/kg b,w.) and group VI (600 mg/kg b,w.) respectively). The methanol extract, will be administered to the rats through oral gavages for a period of 14 days.

Group Treatment

I (Normal control) Normal + No treatment

II (Negative control) DEN + No treatment

III (Positive Control) DEN + Standard Drug (Silymarin 100 mg/kg bw)

IV (Treatment I) DEN + Methanol Extract (200 mg/kg bw)

V (Treatment II) DEN + Methanol Extract (400 mg/kg bw)

VI (Treatment III) DEN + Methanol Extract (600 mg/kg bw)

Collection of Samples

On completion of the experimental period, animals were anaesthetized with diethyl either (2ml/kg). The blood was collected with and without EDTA as anticoagulant.

1. Evaluation of the effect of plant extract on Cancer Induced diethyl nitrosamine.
2. Estimation of liver enzyme markers.

   The biochemical parameters determined are aspartate transaminase, alanine transaminase, alkaline phosphatase (Reitman and Frankel, 1957); total bilirubin (Dangerfield and Finlayson, 1953); total protein (Lowery *et al.*, 1959) and albumin (Doumas, 1971).
3. Evaluation of antibacterial potentials of the methanol extract of *Annona senegalese*.

Antibacterial Assay

Preparation of test samples

The crude extracts of *Annona senegalese* was used in antibacterial assay, the methanol crude extracts. The crude extracts were tested by disc diffusion method on nutrient agar medium as described by (Umaru *et al.*, 2018). Exactly 3 mg of the crude sample was dissolved homogeneity in 3 mL of methanol giving a stock solution of 1000 µg/ mL.

Different volumes from the stock solution were taken, amounted to 50, 100, 250, 500 ppm each, and dissolved in 5 mL of methanol to make final concentration respectively.

Preparation of agar plates

Preparation of agar plates was performed based on method described by (Umaru *et al.*, 2018). Nutrient agar was prepared according to manufacturer's instruction with 14 g of dried agar dissolved in 500 mL distilled water. The agar solution was heated until boiling followed by sterilization in autoclave at 121°C. The agar solution was then poured into a sterile petri plate and allowed to cool down and forming a gel. The plate was divided into eight sections by making a line marking on the outside surface of the plate. The eight sections were for each test samples namely the 50, 100, 250, ppm samples, tetracycline 30 µg (positive control) and methanol (negative control). The plate was sealed using parafilm and kept chilled at 4°C upon bacteria inoculation.

Preparation of bacteria broth

Several selected bacteria were used to evaluate the antibacterial activities of the crude extracts of *Annona senegalese* as obtained from the stock culture provided by Microbiology Laboratory, Federal University Wukari. The nutrient broth was prepared according to manufacturer's instruction, with 2.6 g of the dried broth dissolved in 200 mL distilled water followed by sterilization in autoclave at 121°C. The bacterial was sub-cultured in a 10 mL of broth, each in universal glass bottle for 16 hours inside an incubator equipped with shaker at 37°C (Malesh and Satish, 2008). After 16 hours of incubation, turbidity (optical density/OD) of the bacterial broth was measured by using UV mini spectrophotometer (model 1240 of Shimadzu brand), comparable to that of nutrient broth standard tube for further use. Measurement was performed at wavelength 575 nm and the bacterial broth was ready to be used when its turbidity was between OD 0.6 to 0.9. Nutrient broth was used to adjust the turbidity until the desired value was obtained.

Plate inoculation

Inoculation of the bacteria was carried out in a biohazard cabinet and the procedure was based on method described by (Umaru *et al.*, 2018). Approximately 1 mL of the ready bacterial broth were transferred into mini centrifuge tubes. A sterile cotton swap was dipped into the mini centrifuge tube containing bacteria broth and streaked over entire of the agar plate surface, performed in 4 different directions. The agar plate was then left for 5-10 minutes before applying the test samples. The disc used was 6 mm diameter. A

volume of 10 µL of the test samples of concentration 100,200 and 300 ppm was each pupated onto the discs and placed onto the agar plate by using sterile forceps and gently pressed to ensure contact. Next to be placed on the agar plate was the disc pupated with methanol as negative control, followed by 30 µg of tetracycline as standard antibacterial agent (positive control). The plates were left at room temperature for 10 minutes to allow the diffusion of the test samples and the standards into the agar. Each crude extract was tested in triplicate for each bacterium used. The plate samples were then incubated at 37°C for 24 hours before the inhibition zone around every sample disc being examined. The inhibition zone was measured in diameter to indicate the presence of antibacterial activity for each sample, as compared to the positive control (Silymarin).

RESULTS

Liver Function Tests Result

Table 1 Effect of stem-bark extract of *Annona senegalese* on hepatic marker enzymes and hepatic serum proteins

PARAMETER	NORMAL	NEGATIVE (-ve	POSITIVE (+ve) 100mg/kg bwt	STEM-BARK EXTRACT OF ANNONA SENEGALESE		
				200mg/kg.bwt	400mg/kg.bwt	600mg/kg.bwt
ALT (U/L)	25.69 ± 1.52^{C}	54.97 ± 1.54^{e}	17.40 ± 1.08^{a}	39.31 ± 1.57^{d}	19.99 ± 0.87^{b}	$18.55\pm0.42^{a,b}$
ALP (U/L)	142.25 ± 0.66^{a}	368.70 ± 1.14^{f}	155.33 ± 1.42^{b}	211.26 ± 0.47^{c}	238.39 ± 1.61^{e}	229.17 ± 0.95^{d}
AST (U/L)	91.59 ± 0.59^{f}	69.52 ± 0.88^{c}	63.47 ± 1.0^{b}	39.81 ± 1.20^{a}	84.26 ± 1.64^{e}	74.31 ± 1.41^{d}
T. PROTEIN (mg/dL)	7.50 ± 0.42^{d}	$4.r86\pm0.07^{a}$	7.51 ± 0.18^{d}	$5.07\pm0.20^{a,b}$	5.91 ± 0.19^{c}	$5.47\pm0.41^{b,c}$
ALBUMIN (mg/dL)	6.04 ± 0.26^{d}	3.99 ± 0.13^{a}	5.44 ± 0.07^{c}	3.90 ± 0.28^{a}	4.60 ± 0.30^{b}	4.02 ± 0.10^{a}
T. BILURUBIN (mg/dL)	$1.94\pm0.06^{c,d}$	$1.87\pm0.09^{b,c}$	$1.94\pm0.07^{c,d}$	1.67 ± 0.04^{a}	$1.75\pm0.06^{a,b}$	2.03 ± 0.07^{d}
D. BILURUBIN (mg/dL)	0.83 ± 0.12^{a}	$1.06\pm0.22^{a,b}$	$1.03\pm0.14^{a,b}$	$0.95\pm0.06^{a,b}$	1.26 ± 0.25^{b}	$0.96\pm0.06^{a,b}$

The values were presented as mean±S.E.M (n=3). Values not sharing the same superscript letters are significantly ($p<0.05$) different. Values with the same superscript are not significantly ($p<0.05$) different.

Anti-Bacterial Test Result

Table 2: Effect of *Annona senegalese* stem-bark methanol crude extract (µg/mL) on Gram positive and Gram-negative bacteria in millimeter (mm)

Conc. (µg/mL)	Organism	Tetracycline (30 µg/mL)	Methanol
100µg/mL	*Escherichia coli*	22.34± 0.11	13.13± 0.13
	Staphylococcus aureus	22.56± 0.23	15.15± 0.17
200µg/mL	*Escherichia coli*	22.36± 0.17	18.17±0.14
	Staphylococcus aureus	22.36± 0.23	22.14±0.13
300µg/mL	*Escherichia coli*	22.24 ± 0.19	18.16± 0.12
	Staphylococcus aureus	22.16 ± 0.16	20.17 ± 0.14

Result is Mean ± SD. N = 3

*= significant activity was observed when compared to the control ($p<0.05$).

Concentration of standard is 30 µg/mL of tetracycline, Conc= Concentration

DISCUSSION

Effect of Stem-Bark Extract of *Annona senegalese* on Hepatic Marker Enzymes and Hepatic Serum Proteins

The study investigated the effect of stem-bark extract of *Annona senegalese* on Hepatic Marker Enzymes and Hepatic Serum Proteins including Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Total Protein, Albumin, Total Bilirubin and Direct Bilirubin.

Alanine aminotransferase (ALT) is a key enzyme found primarily in the liver, and elevated levels in the blood are a strong indicator of liver damage or injury. The highest ALT level was observed in the negative control group (DEN-induced) (54.97 U/L) compared to the normal control group (25.69 U/L). The positive control group (DEN + Silymarin) showed a significant reduction in ALT levels (17.40 U/L). Among the treatment groups, the 200 mg/kg extract group showed elevated ALT levels (39.31 U/L), which were higher than the normal control but lower than the negative control. However, the higher

doses of the extract (400 and 600 mg/kg) resulted in a significant reduction in ALT levels (19.99 and 18.55 U/L, respectively). This reduction in ALT levels at higher doses aligns with findings from other studies on plant-based hepatoprotective agents, such as ginger extract, which have also demonstrated dose-dependent reductions in ALT levels in DEN-induced liver damage models (Mansour *et al.*, 2019). The increase in ALT levels at the lower dose (200 mg/kg) suggests that the extract may not be effective at lower concentrations or may exert some stress on the liver, a phenomenon also observed in studies involving *Dicranopteris linearis* leaf extracts at suboptimal doses (Zakaria *et al.*, 2020).

Alkaline phosphatase (ALP) is often elevated in conditions involving bile duct obstruction or liver disease. The negative control group showed a very high level of ALP (368.70 U/L), indicating significant liver damage and possible bile duct obstruction. The positive control group (DEN + Silymarin) showed a reduction in ALP levels (155.33 U/L). The treatment groups also showed elevated ALP levels compared to the normal control (142.25 U/L), but there was a gradual decrease in ALP levels with increasing doses of the extract (211.26 U/L at 200 mg/kg, 238.39 U/L at 400 mg/kg, and 229.17 at 600 mg/kg). The reduction in ALP levels in the treatment groups, especially at 600 mg/kg, indicates that the extract may help in restoring liver function and reducing bile duct obstruction. This partial reduction in ALP levels is consistent with findings from studies on *Phyllanthus amarus*, which have shown similar dose-dependent reductions in ALP levels in liver injury models administered Phyllanthin (You *et al.*, 2021).

Aspartate aminotransferase (AST) is elevated in liver damage, but it is also found in other tissues such as the heart and muscles. The negative control group showed elevated AST levels (69.52 U/L) compared to the normal control (91.59 ± 0.59 U/L). The positive control group (DEN + Silymarin) showed a significant reduction in AST levels (63.47 U/L). Among the treatment groups, the 200 mg/kg extract group showed the most significant reduction in AST levels (39.81 U/L), which was even lower than the positive control. However, the higher doses (400 and 600 mg/kg) showed an increase in AST levels (84.26 and 74.31 U/L, respectively). This biphasic effect, where lower doses (200 mg/kg) are protective but higher doses (400 and 600 mg/kg) may cause mild toxicity, has been observed in other studies involving plant extracts, such as *Garcinia kola* on acute toxicity of male wistar rats (Bolajoko *et al.*, 2019) and *Vernonia amygdalina* and *Azadirachta indica* against acetaminophen-induced hepatotoxicity in Sprague-Dawley male albino (Johnson *et al.*, 2015).

Total protein levels in the blood are an indicator of the liver's synthetic function, as the liver produces most of the proteins in the blood. The negative control group showed significantly lower total protein levels (4.86 ± 0.07 mg/dL) compared to the normal control (7.50 mg/dL). The positive control group (DEN + Silymarin) showed a significant improvement in total protein levels (7.51 mg/dL). Among the treatment groups, the 200 mg/kg extract group showed a slight improvement in total protein levels (5.07 mg/dL), while the higher doses (400 and 600 mg/kg) showed further improvement (5.91 and 5.47 mg/dL, respectively), though they were still lower than the normal control. These findings are consistent with studies on *Phyllanthus amarus*, which have shown similar improvements in serum protein levels in liver injury models (You *et al*., 2021).

Albumin is a specific protein produced by the liver, and low levels are a sign of liver dysfunction. The negative control group showed significantly lower albumin levels (3.99 mg/dL) compared to the normal control (6.04 mg/dL). The positive control group (DEN + Silymarin) showed a significant improvement in albumin levels (5.44 mg/dL). Among the treatment groups, the 200 mg/kg extract group showed no significant improvement in albumin levels (3.90 mg/dL), while the higher doses (400 and 600 mg/kg) showed slight improvement (4.60 and 4.02 mg/dL, respectively).

The reduction in total bilirubin levels at the 200 mg/kg dose suggests that the extract may help in improving liver function, particularly in bile production and excretion (Okaiyeto *et al*., 2018). However, the increase in total bilirubin levels at higher doses (400 and 600 mg/kg) indicates that the extract may exert some stress on the liver at higher concentrations. The negative control group showed elevated direct bilirubin levels (1.06 mg/dL) compared to the normal control (0.83 mg/dL). The positive control group (DEN + Silymarin) showed no significant change in direct bilirubin levels (1.03 mg/dL). Among the treatment groups, the 200 mg/kg extract group showed a slight reduction in direct bilirubin levels (0.95 mg/dL), while the higher doses (400 and 600 mg/kg) showed an increase in direct bilirubin levels (1.26 and 0.96 mg/dL, respectively). The slight reduction in direct bilirubin levels at the 200 mg/kg dose suggests that the extract may help in improving bile duct function. This increase in bilirubin levels at higher doses has also been reported in studies involving ginger extract, suggesting that high doses of plant extracts may exert some stress on the liver (Mansour *et al*., 2019).

Effect of *Annona senegalese* Stem-Bark Methanol Crude Extract (µg/mL) on Gram-Positive and Gram-Negative Bacteria

The antibacterial activity of the methanol stem-bark extract of *Annona senegalese* was evaluated against two types of bacteria: Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*). The results indicate the effectiveness of the extract in inhibiting bacterial growth. The study compared the extract's performance to a standard antibiotic (tetracycline, 30 µg/mL) and a negative control (methanol).

At a concentration of 100 µg/mL, the extract showed moderate antibacterial activity against both *E. coli* and *S. aureus*. For *E. coli*, the inhibition zone was 13.13 mm, which is significantly smaller than the inhibition zone produced by tetracycline (22.34 mm). For *S. aureus*, the inhibition zone was 15.15 mm, compared to tetracycline's the inhibition zone (22.56 mm). This is consistent with findings from studies on other *Annona* species, such as *Annona muricata* and *Annona squamosa*, which have also shown moderate antibacterial activity at lower concentrations (Bhardwaj *et al.*, 2020; Quílez *et al.*, 2018). When the concentration of the extract was increased to 200 µg/mL, the antibacterial activity improved significantly. For *E. coli*, the inhibition zone increased to 18.17 mm, and for *S. aureus*, it reached 22.14 mm, which is nearly comparable to tetracycline's inhibition zone (22.36 mm).

At the highest concentration tested (300 µg/mL), the extract's antibacterial activity showed a slight plateau. For *E. coli*, the inhibition zone was 18.16 mm, which is similar to the result at 200 µg/mL. For *S. aureus*, the inhibition zone was 20.17 mm, slightly lower than the 200 µg/mL result but still significantly higher than the 100 µg/mL result. This suggests that while the extract's effectiveness increases with concentration, there may be a limit to its antibacterial potential at very high doses. This plateau effect has been observed in other studies (Bhardwaj *et al.*, 2020; Quílez *et al.*, 2018)

The stronger antibacterial activity of the extract against *S. aureus* (Gram-positive) compared to *E. coli* (Gram-negative) is consistent with the behaviour of many natural compounds. Gram-positive bacteria, such as *S. aureus*, have a simpler cell wall structure compared to Gram-negative bacteria, making them more susceptible to the action of antibacterial agents (Bhardwaj *et al.*, 2020). The extract's ability to nearly match the performance of tetracycline against *S. aureus* at 200 µg/mL highlights its potential as a natural alternative or adjunct to conventional antibiotics, particularly in the fight against

Gram-positive bacterial infections. The antibacterial properties of the extract are likely due to the presence of bioactive compounds such as alkaloids, flavonoids, tannins, and acetogenins, which are known to have antimicrobial effects. The dose-dependent nature of the extract's activity suggests that higher concentrations of these bioactive compounds enhance its antibacterial efficacy (Bhardwaj *et al.*, 2020).

CONCLUSION

The methanol stem-bark extract of *Annona senegalese* demonstrates significant hepatoprotective and antibacterial properties, effectively reducing liver enzyme markers (ALT, AST, ALP) and improving serum protein levels (total protein and albumin) in rats with DEN-induced liver damage, though higher doses (400 and 600 mg/kg) may slightly stress the liver. The extract also exhibits strong, dose-dependent antibacterial activity against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria, with near-comparable efficacy to tetracycline against *S. aureus* at 200 μg/mL. These findings show the potential of *Annona senegalese* as a natural therapeutic agent for liver protection and bacterial infections, particularly against Gram-positive bacteria.

Recommendations

Based on the findings of this study, the following recommendations are proposed:

i. Further studies should be conducted to determine the optimal dosage of Annona senegalese extract for hepatoprotection and antibacterial activity, while investigating potential toxicity at higher doses.

ii. Detailed phytochemical analysis should be performed to identify and quantify the specific bioactive compounds responsible for the extract's hepatoprotective and antibacterial effects.

iii. The mechanisms by which the extract reduces liver damage and inhibits bacterial growth should be investigated to better understand its therapeutic potential.

iv. Further studies should be done to test the efficacy of the extract against a wider range of bacterial strains, including antibiotic-resistant pathogens like MRSA and multidrug-resistant *E. coli*, to assess its potential as an alternative or adjunct to conventional antibiotics.

v. Clinical trials should be initiated to evaluate the safety and efficacy of the extract in humans, and develop pharmaceutical formulations for easier administration and improved bioavailability in therapeutic applications.

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