

Full Length Research Paper

Antimicrobial activities of the extract of shea tree (*Vitellaria paradoxa* Gaertn. F.) leaf and bark on some selected clinical pathogens

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The use of plants and plant products in the treatment of various ailments has been with humanity since the dawn of time and the potentials of these plants are so enormous that the continuous search for their hidden treasures is more crucial because of the rise in antibiotic resistance. This study was designed to determine the antimicrobial activities of the shea tree (*Vitellaria paradoxa*) extracts against some selected clinical pathogens. Five pathogenic microorganisms, namely, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Escherichia coli*, and *Salmonella typhi* were used to assess the efficacy of the extracts. The antimicrobial effects of the extracts were examined by the agar well diffusion method on the selected pathogens using Mueller Hinton agar. The control used was Amoxicillin antibiotic. The results revealed that both the bark and crude leaf extracts had antibacterial effect on each of the clinical isolates. The crude leaf extract showed the lowest activity against all tested microorganisms, while the bark extract showed the highest. The bark extract recorded the highest zone of inhibition of 15.5 mm. The study recommends shea tree extracts as a potential source of antibiotic substance against infections caused by the tested microorganisms.

Key words: Shea tree, leaf extract, barks extract, crude extract, pathogens.

INTRODUCTION

The increasing number of drug-resistant pathogens necessitates the development of new formulations to combat this threat. Plants serve as reservoirs of bioactive compounds synthesized for defense against microorganisms and herbivores. The potential of these compounds in plants can be harnessed due to their relative cost-effectiveness and environmentally friendly nature. Plants have traditionally been valuable sources of

medicinal compounds, and many modern medicines are derived from them. These bioactive compounds exist in the form of secondary metabolites found in one or more parts of the plant (Adeleke et al., 2018). Given that herbal medicines are biodegradable, safe, and associated with fewer side effects, the screening of plants for bioactive compounds has become increasingly important in today's world (Fbalana et al., 2016). Notably, one of the most

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Common ways of administering herbs to patients is in the form of a liquid or through herbal tea consumption (Fbalana et al., 2016). The shea tree is one of the most common indigenous tree species cultivated in various parts of Africa, and it is known to possess antimicrobial properties (Falana et al., 2015). Native to the Savanna belt in West and East Africa, the shea tree naturally grows from Senegal in the west to Kenya and Uganda in the east (Maanikuu and Peke, 2017; Iddrisu et al., 2019). A long-lived tree native to the Sahel regions of West Africa, the shea tree is a significant source of edible oil and shea butter (Choungo-Nguekeng et al., 2021).

The leaves, bark, and roots of the shea tree are utilized for various medicinal purposes, including healing burns, treating diarrhea, alleviating mouth sores, addressing boils, and acting as a dewormer (Adeola, 2015). In northern Ghana, the leaves are specifically used in medicine to relieve abdominal pain, especially in adolescents. Furthermore, the leaves are incorporated into a traditional blend with other herbs to create a vapor used for bathing, effectively treating fevers and headaches (Ozioma and Chinwe, 2019).

In traditional medicine, the roots, along with the bark, of the shea tree are employed to treat conditions such as jaundice, diarrhea, and abdominal pain. Specifically, the root bark, when pounded and boiled, is used to heal stubborn wounds in horses (Builders and Builders, 2016). In certain communities, shea tree bark is boiled and consumed as a locally brewed drink, believed to have properties that can treat diabetes. Infusions made from the bark have been historically used to address a variety of ailments, including dysentery, diarrhea, gastrointestinal disorders, and leprosy (Omara et al., 2020). Breastfeeding mothers also consume this brew as it is believed to enhance milk flow. Moreover, it is thought to possess the ability to counteract the venom of the spitting cobra, leading to the use of a bark infusion as eyewash (Adeola, 2015). In some Ghanaian communities, shea tree is used as a foot bath to remove jiggers (Maanikuu and Peke, 2017).

Plants have long served as a source of medication to address pathogenic illnesses, and herbal medicine has been utilized across various cultures and civilizations to treat infections (Inusa et al., 2018). In recent times, the increasing antibiotic resistance of clinically important pathogens has led to the emergence of bacterial strains resistant to many commonly used antibiotics. The issue of antimicrobial resistance has renewed interest in herbal or medicinal plant products as potential sources of new compounds to address the growing problem of drug resistance.

The high cost and scarcity of newly invented antibiotics in developing countries contribute to an increasing mortality rate in recent times, as they are beyond the reach of ordinary citizens (Falana et al., 2015). Therefore, this study aimed to determine the antimicrobial activities of shea tree (*Vitellaria paradoxa* Gaertn. F.) extracts against some selected clinical pathogens.

METHODOLOGY

Sample collection from the tree species

Bark and leaf samples of shea trees were collected from the parklands of Navrongo in the Upper East Region of Ghana. The tree species were identified and authenticated with the assistance of a trained taxonomist. In the parklands, 30 individual matured trees (that is, trees that have been producing fruits for at least the last five years) were randomly selected. The barks of the trunk, located above 1.5 m (that is, at breast height) from the ground, were scraped, with an average fresh weight of 20 g per tree. Leaf samples were also collected from the same trees, with an average weight of 20 g per tree. Fully developed leaves were obtained from the middle branches of the trees. The bark and leaf samples were then thoroughly mixed separately for all 30 trees. Each component was subsampled into sterile polythene bags and transported to the laboratory. The samples were cut into smaller pieces, placed in trays, and exposed separately at room temperature (that is, temperature and relative humidity of $25\pm 2^\circ\text{C}$ and $72\pm 5\%$, respectively) under shade in the laboratory for 7 days.

Collection of pathogens

The pathogens were obtained from the microbiology laboratory of the Department of Applied Biology, C. K. Tadam University of Technology and Applied Sciences in Navrongo, Upper East Region, Ghana. The authentication of these pathogens was conducted by the Kumasi Centre for Collaborative Research (KCCR). Preserved specimens from their storage containers were used for the collection. The collected pathogens included *Candida albicans*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus cereus*, and *Escherichia coli*.

Sterilization

To ensure aseptic conditions, all items were cleaned, air-dried, and sterilized in an autoclave. The workbenches were disinfected with 70% ethanol. Pipettes, pipette tips, conical flasks, and beakers underwent aseptic sterilization in an autoclave for 15 min at 121°C . Petri dishes and spatulas were additionally sterilized in a hot air oven for 24 h at 160°C , and stem borers were sanitized using a Bunsen burner before and after each use. Prior to incubating samples, the incubator was sterilized with 70% ethanol.

Sample preparations

The leaves and bark of the shea tree were thoroughly washed in distilled water to remove dirt and unwanted particles. Subsequently, the leaves and bark samples were dried on sterile plates in the laboratory at room temperature. The dried leaves and bark of the plants were then pulverized into a powder using a sterilized mortar and pestle, and the resulting powder was stored in a sterile container for later use.

Preparation of hot aqueous extracts

Ten grams of fine powder obtained from the bark and leaves of the shea tree were weighed and placed in separate conical flasks, each appropriately labeled. In each conical flask, 90 ml of distilled water was added and heated in a water bath until it reached boiling point. Subsequently, after boiling, the mixture was allowed to cool to room temperature with frequent agitations. Following this, each extract was sieved using muslin cloth and then filtered through Whatman

No. 1 filter paper. The filtered extracts were concentrated and air-dried at room temperature. The dried material was stored in a sterile McCartney container at 4°C until use.

Preparation of ethanol extracts

Ten grams of fine powder extracted from the leaves and bark were placed in separate conical flasks and labeled. Subsequently, 90 ml of 70% ethanol was added to each conical flask, and the mixture was left at room temperature for 24 h with regular agitations to allow the ethanol to evaporate. Distilled water was then added to the flasks. After sieving with muslin cloth, the extracts were further filtered using Whatman No. 1 filter paper, concentrated, and heat-dried at room temperature (Ingle et al., 2017). The resulting dried material was stored in a sterile McCartney container and refrigerated at 4°C until use.

Preparation of nutrient broth

The media were prepared following the manufacturer's instructions. In a conical flask, 25 g of nutritional broth was suspended in 1000 ml of distilled water and thoroughly mixed to achieve a uniform solution. The solution was then heated for 30 min to ensure complete dissolution of the media. Subsequently, the medium was set aside to cool. The test organisms were then placed in separate labeled test tubes. The test tubes containing the different organisms were arranged in a randomized complete block design with three replications.

Preparation of Mueller-Hinton

The agar was prepared following the manufacturer's instructions. In a conical flask, 28 g of powdered Mueller Hinton agar was weighed and mixed with 1000 ml of distilled water. The mixture was stirred and heated to ensure thorough dissolution of the medium. Subsequently, the medium was autoclaved at 121°C for 15 min before being dispensed onto several Petri dishes.

Preparation of McFarland standard No. 0.5

As a reference, a 0.5 McFarland solution was prepared to adjust the turbidity of the bacterial suspension. Barium chloride (0.5875 g) was weighed and dissolved in 50 mL of distilled water. Additionally, 100 mL of distilled water was mixed with 1 mL of sulfuric acid and thoroughly shaken. Subsequently, 0.5 mL was pipetted off, and 0.5 mL of barium chloride was added to create a turbid solution. The prepared solution was then kept at room temperature until needed.

Preparations of clinical isolates

For the clinical isolates, 15 ml of sterile nutrient broth was poured into each test tube, and 0.1 ml of each pathogen inoculum was pipetted into the test tubes containing the media. The tubes were then incubated at 37°C for 18 to 24 h to allow the pathogens to grow. Growth was indicated by a hazy medium.

Preparation of inoculum

Inoculum preparation using McFarland standard No. 0.5 was performed. A day-old isolate was obtained by cultivating a pure culture of clinical pathogens in nutritional broth and incubating it at

37°C for 18 to 24 h. Additionally, 1 ml of clinical isolates was distributed and plate-cultured on sterile Mueller Hinton agar, allowed to set. To create an agar well on the plate, a 6 mm sterile cork borer was utilized. Subsequently, 0.1 ml of each extract was placed in an agar well on the plate. The plates were left on the benches to allow absorption of the extracts before being flipped and incubated at 35 to 37°C for 24 h. The zone of inhibition was measured and recorded afterward.

Antibiotic preparation used as control

For the control, 250 mg of an antibiotic, Amoxicillin, was dissolved in 25 ml of distilled water. Amoxicillin was employed as a positive control. This antibiotic was used as a control because it is widely used in Navrongo and its environs and is considered to be most effective among many others. The antibiotic dissolved in distilled water was placed in control wells.

Antimicrobial sensitivity test

The antibacterial activities of ethanol extract and hot aqueous extract from the leaves and bark of the shea tree were determined using the Agar well diffusion technique. For all extracts, Mueller-Hinton agar plates were prepared, and 0.1 ml inoculums of specified human pathogens were dispensed using a pipette over the agar surface and uniformly distributed using swap sticks. After 5 min, a 6 mm diameter cork borer was used to drill wells, and equal amounts (0.5 ml) of each extract were placed into each well on the agar. Amoxicillin was poured into the corresponding wells. The plates were allowed to stand for 12 h to allow the extracts and Amoxicillin to thoroughly diffuse into the agar.

Subsequently, the plates were incubated upside down at 37°C for 24 h, and the zones of inhibition were measured using a ruler.

Data analysis

The results are expressed as means \pm standard deviations. The data were analyzed using one-way analysis of variance (ANOVA), and in cases of significant differences, the Tukey Post Hoc test was employed to differentiate between the means. The Zone of Inhibition Diameter for Amoxicillin (Control) was utilized to further analyze the data, following the criteria described by Mcdermott et al. (2008): Resistant = \leq 11 mm; Intermediate, 11 to 15 mm; and Susceptible/Sensitive \geq 15 mm.

RESULTS

The effect of hot aqueous and ethanol extracts of bark of shea tree on selected pathogens

The antimicrobial activities of ethanol and hot aqueous extracts of the bark of the shea tree are presented in Table 1. The results revealed that *B. cereus* (15.5 mm) was sensitive to the ethanol extract of the bark, while *P. aeruginosa* (13.5 mm), *C. albicans* (12.0 mm), and *E. coli* (11.0 mm) showed intermediate sensitivity to the ethanol extract of the bark. *S. typhi* (10 mm) was resistant to the ethanol extract of the bark. Therefore, *B. cereus* exhibited the maximum zone of inhibition, while *S. typhi* had the least zone of inhibition with the ethanol extract of the

Table 1. Antimicrobial effects of bark extracts of shea tree and antibiotic on selected clinical isolates.

Sample/Pathogen	Zones of inhibition (mm)				
	<i>B. cereus</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>E. coli</i>	<i>S. typhi</i>
Ethanol extract (bark)	15.5 ^a ±0.71	13.5 ^a ±0.71	12.0 ^a ±1.41	11.0 ^a ±0.00	10.0 ^a ±1.41
Hot aqueous (bark)	12.5 ^a ±0.71	14.5 ^a ±0.71	11.0 ^a ±0.00	15.0 ^b ±0.00	11.0 ^a ±1.41
Amoxicillin	28.0 ^c ±2.83	23.5 ^c ±0.71	25.5 ^c ±0.71	25.5 ^c ±0.71	25.5 ^c ±0.71

Mean values in the same column followed by different superscripts (letters) are significantly different (Tukey, $P \leq 0.5$).

shea tree bark.

The results also indicated that *E. coli* (15.0 mm) was sensitive to the hot aqueous extract of the bark, but the other clinical pathogens, namely, *B. cereus* (12.5 mm), *P. aeruginosa* (14.5 mm), *C. albicans* (11.0 mm), and *S. typhi* (11.0 mm), showed intermediate sensitivity to the hot aqueous extract of the bark used in this study.

Despite the varying levels of sensitivity exhibited by the clinical pathogens to the shea tree extracts, the bark extracts of both ethanol and hot aqueous did not demonstrate any significant differences ($p > 0.05$) in the zone of inhibition for four of the clinical pathogens. *E. coli* is the only clinical pathogen whose zone of inhibition showed a significant difference ($p < 0.05$) between the ethanol and hot aqueous extracts of the shea tree bark. Furthermore, both the ethanol and hot aqueous extracts of the bark significantly ($p < 0.05$) underperformed compared to the control (Amoxicillin) antibiotic in the zone of inhibition for all five clinical pathogens used in this study.

The effect of hot aqueous and ethanol extracts of leaves of shea tree on selected pathogens

The results of this study indicated that all the clinical pathogens used, namely, *B. cereus* (11.0 mm), *P. aeruginosa* (11.5 mm), *C. albicans* (13.0 mm), *E. coli* (12.05 mm), and *S. typhi* (13.0 mm), showed intermediate sensitivity to the ethanol extract of the leaf of the shea tree.

Furthermore, the results also indicated that four of the pathogens used, namely, *B. cereus*, *P. aeruginosa*, *C. albicans*, and *S. typhi*, showed resistance to the hot aqueous extract of the leaf of the shea tree. The results further showed that only *E. coli* (12.0 mm) exhibited intermediate sensitivity to the hot aqueous extract of the leaf of the shea tree (Table 2).

Additionally, the results indicated that the ethanol and hot aqueous extracts of the leaf of the shea tree showed no significant differences ($p > 0.05$) in the zone of inhibition of *P. aeruginosa* and *E. coli*. Nevertheless, the results showed significant differences ($p < 0.05$) in the zone of inhibition of the other three pathogens, namely, *B. cereus*, *C. albicans*, and *S. typhi*. Once more, the Amoxicillin antibiotic used as a control significantly ($p <$

0.05) outperformed both the ethanol and hot aqueous leaf extracts of the shea tree in the zone of inhibition of all the clinical pathogens used in this study.

DISCUSSION

The plant extract exhibits antibacterial properties against a variety of microorganisms, according to Mohammed and Omar (2015). The bark and leaf extracts showed antibacterial activity against all the pathogens. The leaf extracts had the least effect against four of the organisms tested, namely *B. cereus*, *P. aeruginosa*, *C. albicans*, and *S. typhi*. The zone of inhibition was largest in the bark extract. According to Obioma et al. (2017), the bioactive compounds of *V. paradoxa*, such as glycosides, tannins, saponins, carbohydrates, alkaloids, and terpenoids present in plant parts, have been shown to be responsible for the antimicrobial activity of medicinal plants, though steroids were absent in the bark while leaf extracts lack polyphenols. The increased antimicrobial activity in the bark may be attributable to the presence of polyphenols that have been reported to be absent from the shea tree leaf (Abubakar, 2010). However, *E. coli* exhibited intermediate sensitivity to the use of the hot aqueous extract of the leaf. It may be difficult at this moment to assign any cogent reason why this happened, but the phytochemical composition in all parts of *V. paradoxa*, such as the root, leaf, and bark, contains tannins, glycosides, saponins, carbohydrates, alkaloids, and terpenoids (Mathias et al., 2007). It may be attributable to different concentrations of the biochemicals in the leaf and the bark. It has also been reported that the seeds and stem bark are deficient in steroids (Obioma et al., 2017). Moreover, these phytochemicals could be primarily contributed by the tree parts rather than the soil because the study area soils comprised predominantly of Savanna ochrosols that contain chemical constituents such as nitrogen, phosphorus, potassium, calcium, cation exchange capacity, among others (Imoro et al., 2017). However, these chemical constituents could serve as precursors in the preparation of the aforementioned phytochemicals in the tree parts. Also, the kind of solvent utilized for the extraction may have had an impact on the antibacterial activity of the three extracts based on the existence and

Table 2. Antimicrobial effects of leaf extract of shea tree and antibiotic on selected clinical isolates.

Sample/pathogen	Zones of inhibition (mm)				
	<i>B. cereus</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>E. coli</i>	<i>S. typhi</i>
Ethanol extract (leaf)	11.0 ^a ±0.00	11.5 ^a ±0.71	13.0 ^a ±0.00	12.5 ^a ±0.71	13.0 ^a ±1.41
Hot aqueous (leaf)	0.00 ^b ±0.00	10.0 ^b ±0.00	0.00 ^b ±0.00	12.0 ^a ±0.00	0.00 ^b ±0.00
Amoxicillin	28.0 ^c ±2.83	23.5 ^c ±0.71	25.5 ^c ±0.71	25.5 ^c ±0.71	25.5 ^c ±0.71

Mean values in the same column followed by different superscripts (letters) are significantly different (Tukey, $P \leq 0.5$).

concentration of secondary metabolites (Liu et al., 2016). The hot aqueous extract of the bark presented a higher zone of inhibition for *E. coli* than that of the ethanol extract of the bark in this study. This implies that the aqueous extract of the bark of the shea tree exhibited higher antibacterial activity against *E. coli* than the ethanol extract on the same pathogen. This probably could arise because the water might have extracted more of the phytochemicals that inhibit *E. coli* better and thus presented a higher zone of inhibition for the aqueous extract. This agrees with an earlier study by Caleja et al. (2016), who found hot water extracts to yield significantly higher amounts of phytochemicals than ethanol extracts in some plants. However, Ndukwe et al. (2005) reported better antimicrobial activity in some microorganisms with ethanol extracts than hot aqueous extracts of some chewing sticks (plants). In general, tannins seem to be promising antibacterial and antiviral substances for some bacterial diseases (Farha et al., 2020). The antibacterial activity of the ethanol extracts of *V. paradoxa* is organism-dependent. The physiological properties of the clinical isolates, as well as the presence or absence of some active ingredients in the extracts, may account for differences in the sensitivity of the organisms tested and variations in the specific activity of each extract (Arekemase et al., 2013). Higher concentrations of antimicrobial substances have been shown to have detectable antimicrobial activity (Arekemase et al., 2013). The inability of the aqueous extracts to inhibit some of the organisms may be due to the presence of negligible active components required to inhibit the growth of the test organisms. Plants contain a large number of complex chemical substances of different compositions, which occur as phytochemicals in one or more parts of the plant known to be responsible for the antimicrobial activity of medicinal plants (Ganesh et al., 2007). In particular, saponins have been reported to have more potent antimicrobial activity and act as precursors of steroid substances that have a broad spectrum of physiological activities (Pacheco-Ordaz et al., 2017). Tannins have been reported to have astringent and detergent properties (Zhu et al., 2019), suggesting they could be effective as an anti-fungal agent. Thus, the study showed the effects of both the ethanol and hot aqueous extracts of the leaf and bark of the shea tree on *C. albicans*.

In general, lignin is commonly found in plant biomass and has a tremendous inhibiting effect on several pathogenic organisms. The study of metabolomics-based plant phytochemicals has revealed the antimicrobial activities of lignin. Indeed, recent research found that agricultural residues like pepper and coffee husk extracts have high antibacterial activity, and the analysis of their bioactive functions implicated lignin and phenol derivatives (Verrillo et al., 2021).

Tannins have also been shown to inhibit bacterial growth through a variety of mechanisms, including iron chelation, inhibition of cell wall synthesis, destruction of the cell membrane, and inhibition of fatty acid synthesis (Romaniuk and Cegelski, 2015; Pacheco-Ordaz et al., 2017). Tannins can inhibit quorum sensing and reduce gene expression of virulence factors such as biofilms, enzymes, adhesions, motility, and toxins. Nanoparticles/hydrogels loaded with tannins also have antibacterial properties. Overall, tannins appear to be promising antibacterial and antiviral agents for the prevention of bacterial infections (Farha et al., 2020). Additionally, the differences in the sensitivity of the organisms tested and variations in the specific activity of each extract may be due to the physiological properties of the clinical isolates and the presence or absence of some active ingredients in the extracts (Arekemase et al., 2013). The inability of the aqueous extracts to inhibit some of the organisms may be due to the presence of negligible active components required to inhibit the growth of the test organisms. Plants contain a large number of complex chemical substances of different composition, which occur as phytochemicals in one or more parts of the plant. Consequently, the ethanol and aqueous extracts of both the bark and the leaf of the shea tree exhibited some amount of antimicrobial activities on the tested microorganisms, and these plant parts could be a good alternative in the treatment of the infections caused by those pathogenic organisms used in this study. The differences in the types and concentrations of phytochemical ingredients, as well as the percentage of extract yield, may be caused by the different solvents' capacities for soluble substances. A substance's different solubilities may depend on the physical and chemical characteristics of the solvents used and the phytochemicals that make them up. Types, quantities, and interactions of secondary metabolites found in

extracts and fractions are thought to be determinants of antibiotic activity (Felhi et al., 2017). The local use of these herbs in the treatment of specific illnesses is supported by the evidence of action against the test microorganisms. This finding is important because it may lead to the creation of medications that are effective against organisms that are multidrug-resistant (Terreni et al., 2021).

Conclusion

This study found that *B. cereus* showed sensitivity to the ethanol extract of the bark, and *P. aeruginosa*, *C. albicans*, and *E. coli* showed intermediate sensitivity to the ethanol extract of the bark. *S. typhi*, however, exhibited resistance to the ethanol extract of the bark. The study also found that *E. coli* was sensitive to the hot aqueous extract of the bark, and the other clinical pathogens used in this study showed intermediate sensitivity to the hot aqueous extract of the bark of the shea tree. The study further concludes that all the clinical pathogens used exhibited intermediate sensitivity to the ethanol extract of the leaf, while four of the pathogens, namely, *B. cereus*, *P. aeruginosa*, *C. albicans*, and *S. typhi*, showed resistance to the hot aqueous extract of the leaf of the shea tree.

Recommendation

The study, therefore, recommends that since both ethanol and hot aqueous extracts inhibited the test organisms to some extent, the bark of the shea tree could be used in traditional medicine to treat a variety of diseases associated with the tested isolates. Indeed, it provides a potential and less expensive source for antibiotic discovery and manufacture.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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