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Research Article

DETERMINATION OF BIOACTIVE COMPOUNDS AND BIOLOGICAL PROPERTIES OF *TAMARINDUS INDICA* STEM BARK EXTRACT AGAINST SELECTED MICROBES

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ABSTRACT

Objectives: The present study determines the bioactive constituents in the stem bark of *Tamarindus indica* and also assesses its biological properties against *Staphylococcus aureus* and *Pseudomonas aeruginosa in vitro*.

Methods: The clinical isolates were collected from wounds of patients and cultivated in a nutrient broth. The extract of stem bark of *T. indica* was added to the bacteria in Muller Hinton ager to test its biological activity.

Results: Crude extracts of *T. indica* showed potency against *S. aureus* with the highest zone of inhibition of 19.00 ± 0.60 mm at 200 mg/ml concentration of the extract. The extract showed potency against *P. aeruginosa* at varying concentrations with a zone of inhibition between 13.5 ± 0.20 mm and 6.25 ± 0.45 mm. However, the extracts of *T. indica* recorded a minimum inhibitory concentration (MIC) value of 6.25 mg/ml and an minimum bactericidal concentration value of 25 mg/ml against *P. aeruginosa* and also recorded a MIC value of 3.13 mg/ml against *S. aureus*. The preliminary analysis of bioactive compounds of the crude extract of *T. indica* showed that it has a wide range of phytochemical constituents including saponins, alkaloids, flavonoids, tannins, and anthraquinones.

Conclusion: The results suggested that the extract of stem bark of *T. indica* has the potential of being the best alternative antimicrobial agent use to cure infectious diseases caused by *S. aureus* and *P. aeruginosa.*

Keywords: Bacteria, Therapeutic, Potency, Antioxidant.

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INTRODUCTION

Medicinal plants are sources of many organic compounds and 70–90% of people living in rural areas depend on herbal medicine for their health care needs [1,2]. Many plants are used by traditional healers due to their antimicrobial activities, which are supported by compounds synthesized as a secondary metabolite of the plant [3]. The biological property of medicinal plants differs with the altitude, climate type, and geographical location from where the plant grows [4]. Globally, the search for new therapeutic agents that are safe, cheap, effective, and widely available is on the rise since most microbes are becoming resistant to synthetic drugs [5]. Extracts of the medicinal plants offer the best alternative to help curb the menace of infectious disease as they contain a wide range of chemical compounds [6]. Bacteria pathogens and other microbes that are multi-drug resistant can be destroyed or their growth inhibited by the use of either crude or refined extracts of medicinal plants [6].

Tamarindus indica is a plant from the legume family which is mostly found in Africa and the tropical zones. The stem bark of *T. indica* was found to contain bioactive molecules such as tannins, saponins, sesquiterpenes, alkaloids, and phlobatannins [7]. Studies conducted on the major plant parts in terms of their pharmacological activity indicate that they have antimicrobial activities and are also capable of destroying fungi [8]. Leaves and stem bark of *T. indica* are used as decoction mixed with potash to relieve stomach disorder, general body pains, jaundice, yellow fever, and skin cleanser in Northern Nigeria [9]. A study on the leaves of T. *indica* reported that it contains 13 essential oils where benzyl benzoate and limonene are major compounds [10]. Gomathi *et al*. [11] reported that pharmacological activities such as antidiabetic, antibacterial, and antioxidants are found in the seeds of *T. indica*. Over the years, scientists have made attempts to develop new drugs that are potent against infectious diseases [12]. However, most studies have been conducted on the leaves and seeds of *T. indica* but our research will based on the stem bark of the plant. Also, previous studies on the plant used either water or methanol as the solvent for extraction and the uniqueness of our study will be the use of acetone. The aim of this research was to determine the bioactive constituents in the stem bark of the plant and also assess the biological properties of *T. indica* that made it widely used for the treatment of wounds and other infectious diseases.

MATERIALS AND METHODS

Plant sample collection

The *T. indica* plant sample was collected in November 2019 from the Tono Irrigation project in Navrongo, a suburb of the Upper East region in the Northern part of Ghana. The plant was further identified by a Botanist, Dr. Isaac Sackey in the University for Development Studies at the Department of Applied Biology.

Plant extraction

Samples of stem bark of T. *indica* were oven-dried at 35°C for 2 days and ground into powder using a mechanical grinder. About 1500 ml of acetone was used to soak 150 g of the ground plant. The mixture was shaken for 30 min. A filter paper was used to filter the extract after 48 h. With the help of a rotary evaporator, the filtrate was concentrated to obtain an 8.80% yield of the extract.

Origin of bacterial

Test bacterial isolates were obtained from wounds of patients who visited the Tamale Teaching Hospital. The isolates *Pseudomonas aeruginosa* and *Staphylococcus aureus* were collected from the Microbiology Department of the hospital in May 2020. In a nutrient broth, the test bacteria were stored at a temperature between 2 and 8°C.

Preparation of crude extracts concentration
Dimethylsulfoxide (DMSO) solvent was used to prepare the concentrations of the crude extract obtained. 0.4 g of the extract was added to 1 ml of DMSO in transparent bottles which have been sterilized to obtain the stock solution of concentration, 400 mg/ml. Concentrations such as 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.13 mg/ml, were derived through serial dilutions.

Bioactive compounds determination
The crude extract obtained was tested for the presence of reducing sugar, flavonoids, alkaloids, Anthraquinones, saponins, tannins, and carbohydrates using the methods described by Trease and Evans [13], Harborne [14], Harbone [15].

Test for reducing sugar

Fehling's test
About 1 ml of the extract was placed in a test tube and diluted with distilled water. Fehling's solution was added and the mixture was heated in boiling water for 5–10 min. The result was observed.

Test for flavonoids

Alkaline reagent test
About 1 ml of extract is added to 1 ml aqueous NaOH and the appearance of yellowish color shows the presence of flavonoids.

Test for alkaloids

Wagner's test
A Wagner's reagent was added to 1 ml of extract. The formation of a brown precipitate indicates the presence of alkaloids.

Test for anthraquinone

Borntrager's test
About 1 ml of extract was added to 5 ml of HCl and the mixture is heated for 10 min. The mixture is filtered; benzene, and an equal amount of ammonia solution were added to the filtrate and shake. The appearance of red color is an indication that anthraquinone is present.

Test for saponins

Foam test
About 1 ml of the extract was added to 10 ml of distilled water and shaken well. The generation of froth indicates the presence of saponins.

Test for tannins

Ferric chloride test
About 5 ml of ferric chloride solution was added to 1 ml of extract. The mixture was observed and the formation of dark green color was an indication that tannins were present.

Test for carbohydrate

Benedict's test
About 1 ml of extract was treated with Benedict's reagent and the mixture was heated gently in a water bath. The result was observed.

Standardization of inoculums
Few colonies of the isolates were left to grow overnight and distributed in sterile normal saline solution such that the turbidity of the cultured isolates will be compared with 0.5 McFarland standards for sensitivity tests as described by the National Committee for Clinical Laboratory Standards [16].

Agar diffusion bioassay
The biological property of *T. indica* extract was determined by using the agar well diffusion method [17]. Sterile Petri dishes were filled with prepared Molten Mueller Hinton (OXOID, Basingstoke, and Hampshire, England) and allowed to solidify. Pure isolates of each bacterium in normal saline solution were dispensed on the solidified agar in the Petri dishes. A sterilized cork borer was used to create wells in the dried Mueller Hinton agar. Moreover, the wells were topped up with 1.0 ml of the different concentrations of the plant extract. Chloramphenicol and DMSO were applied as positive and negative controls respectively. The plates were allowed to stand for 30 min for diffusion to occur and then finally kept in an incubator at 37°C for 24 h. The inhibition zones were measured using a ruler and recorded in millimeters (mm). All the experiments were carried out in triplicate.

Statistical analysis
The data on inhibition zones were analyzed using one-way ANOVA. Each experiment was done in triplicates and the mean and standard error were computed. These means were compared using statistical software to determine if they were significantly different at $p<0.05$.

Assessment of minimum inhibitory concentration (MIC)
To estimate the MIC of the extract for each isolate, we employed the agar well diffusion method. The concentrations, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.13 mg/ml, 1.56 mg/ml, and 0.78 mg/ml of the crude extract were obtained through serial dilution of the stock solution. Molten Mueller Hinton agar was prepared and kept in the Petri dishes and allowed to solidify. A saline solution that contains the test organisms was spread on the solidified agar with the help of a sterilized loop spreader. The wells were created (6 mm deep) by the cork borer and 1.0 ml of the various concentrations of the plant extract were emptied into the wells. Both the negative control (99% DMSO) and the positive control (chloramphenicol) were also drawn into wells each on the same plate. The plates were allowed to stand for 30 min and incubated in inverted positions at 37°C for 24 h. The inhibition zone that was visible enough to be measured as a result of the lowest concentration of the extract that was able to prevent the growth of the bacterial, was recorded as the MIC.

Assessment of minimum bactericidal concentration (MBC)
The MIC was determined by using the tube diffusion method [18]. The MBC of the *T. indica* extract was obtained by using a sterilized transparent bottle for each test organism. A micropipette was used to pick 2 ml of the saline solution, which contains the test organisms and is introduced into the transparent bottles. 1 ml of the various concentrations of the plant extract was dropped into the transparent bottles containing the test organisms. The transparent bottles were corked and kept in an incubator at 37°C for 24 h. The test organisms from each of the bottles were introduced into the nutrient agar plates and the plates were further incubated at 37°C for 24 h. The MBC was also recorded as the lowest concentration that has killed all bacteria on the plate such that no growth of the bacteria is observed.

RESULTS AND DISCUSSION

In rural communities, people treat all kinds of ailments daily using plant extracts. Most of these ailments occur due to the activity of pathogenic organisms. Conventionally, in northern Ghana, the extract of stem bark of *T. indica* is used as an antibiotic for the treatment of wounds. However, medicinal plants are commonly known throughout the world due to their therapeutic properties. The search for a cheap, effective, and less harmful antibiotic from plants against resistant bacterial is a concern for researchers throughout the world [19].

Table 1: Results of bioactive compound determination of stem bark of *Tamarindus indica* extract

Bioactive compounds	Tests	Results
Alkaloids	Wagner's test	Positive
Flavonoids	Alkaline reagent test	Positive
Tannins	Ferric chloride	Positive
Anthraquinoness	Borntrager's test	Positive
Saponins	Foam test	Positive
Reducing sugar	Fehling's test	Negative
Carbohydrates	Benedict's test	Negative

The result of this research revealed that the plant extract contains secondary metabolites including saponins, tannins, alkaloids, flavonoids, and anthraquinones as shown in Table 1. However, reducing sugar and carbohydrate were undetected in the plant extract and this may be due to the solvent used for the extraction. On contrary, Idu and Osadolo [20] reported that the ethanol root extract of *T. indica* saponin and anthraquinone were undetected. Gomathi *et al*. carried out a study on *T. indica* and reported that carbohydrate was undetected in the aqueous extract of *T. indica* [11]. The plant extract was found to contain phytochemicals such as saponins, flavonoids, and alkaloids which conforms to the work of Uchechukwu *et al*. [1]. Najafi's research reported that the presence of bioactive compounds and aromatic secondary metabolites can serve as a natural defense mechanism to protect the plant against pathogens and insect predators [21]. Alkaloids play a key role in metabolism and control development in living organisms and also impede cell division. The antibacterial activity of T. *indica* may be a result of the presence of alkaloids [22].

The biological property of the plant extract was assessed at varying concentrations ranging from 0.78 mg/ml to 200 mg/ml. The crude extract of the plant was found to be potent against all the pathogens used in the study. However, the highest concentration of the extract, 200 mg/ml showed the highest zone of inhibition of 19.00 ± 0.60 mm against *S. aureus* and at the same concentration showed a zone of inhibition of 13.50 ± 0.20 mm against *P. aeruginosa* as shown in Fig. 1. The studies revealed that the plant extract is potent against both groups of bacteria but however its potency was seen to be higher in *S. aureus*, which is a gram-positive bacterium than in *P. aeruginosa,* which is also a gram-negative bacterium. These findings are said to be consistent with those reported by Nascimento [3]. The phytochemicals such as flavonoids, tannins, alkaloids, and anthraquinones were present in the plant extract and these phytochemicals may contribute to the biological activity of the extract against both isolates [23].

The MIC value of the extract against *S. aureus* is recorded as 3.13 mg/ml and the MBC value against *S. aureus* was undetected. In another study that determined the antibacterial activity of the extract of T. *indica* seed pods recorded a MIC of 0.5 mg/ml against *S. aureus* [24] which is similar to the MIC of this study. The MIC and MBC values of the extract against *P. aeruginosa* were 6.25 mm/ml and 25 mm/ml respectively as seen in Table 2. The MIC values of the extract against both bacteria were in agreement with research findings reported by Uchechukw *et al*. [1].

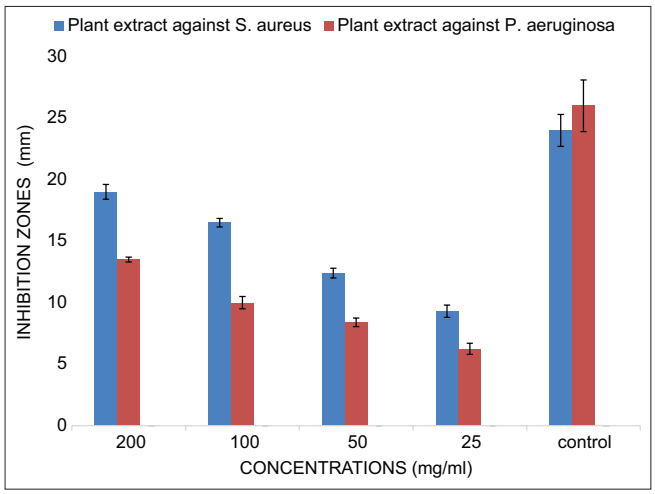

Fig. 1: Biological property of the plant extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Table 2: Minimum inhibition concentration and minimum bactericidal concentration of the plant extract

Plant extract	*Staphylococcus aureus*		*Pseudomonas aeruginosa*	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Tamarindus indica	3.13	UD	6.25	25

UD: Undetected, MBC: Minimum bactericidal concentration, MIC: Minimum inhibition concentration

CONCLUSION

This research revealed that the acetone extract of *T. indica* contains bioactive molecules such as alkaloids, tannins, saponins, flavonoids, and anthraquinones. The presence of these molecules indicates that *T. indica* has the enormous potential of been an antimicrobial agent. The result from the study showed that the extract of *T. indica* is potent against both bacteria but more effective against *S. aureus* and this goes to support the use of the plant extract in Northern Ghana and other countries to treat all manner of ailments. We recommend that further research should be conducted on the plant using different solvents for extraction. Furthermore, more studies are required on the Bioactivity-guided isolation and chemical characterization of the bioactive molecules which are responsible for the biological properties of the plant extract.

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AUTHOR'S CONTRIBUTION

Aboko J and Anabia M carried out the experiment and drafted the manuscript. Acheampong C analyzed the results.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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The cost of the study was incurred by the authors.

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