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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF TOKAR SHA AGAINST ENTERIC BACTERIA

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Abstract

The antibacterial activity of Tokar sha; a local traditional medication widely used by many people in North-west zone of Nigeria especially Sokoto, Kebbi and Zamfara against enteric infections were examined against some clinical isolates of pathogenic bacteria (Staphylococcus aureus, Escherichia coli, Bacillus cereus and Salmonella typhi) using agar well diffusion method. The pattern of inhibition varied with the tokar sha concentrations and the organisms tested. The tokar sha was more effective on E. coli with a maximum zone of growth inhibition of 25mm at 35mg/ml followed by B. cereus (20mm). However, S. aureus and S. typhi were resistant to tokar sha at all concentrations tested. The minimum inhibitory concentrations (MIC) were found to be 35mg/ml for both E. coli and B. cereus. The antibacterial activities exhibited by tokar sha in this study could be

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attributed to the presence of its constituents which signifies the potential of the tokar sha as a therapeutic agent. These findings may justify the ethnomedicinal use of tokar sha as an antibacterial agent against enterobacteria.

INTRODUCTION

Plant-derived medicines have been used due to the significant healing power of the traditional medicinal systems (Adebolu and Oladimeji, 2005). Medicinal plants are distributed worldwide but they are most abundant in tropical countries (Naqvi *et al*., 1991; Elvin-Lewis, 2001). The abundance of medicinal plants in nature and the traditional knowledge increase the understanding of the medicinal plants' properties, safety and efficacy (Nascimento *et al*., 2000). This concern has been expressed because of the resistance of clinically pathogenic microorganisms to the antibiotics that have been produced in the last decades (Cohen, 1992; Nascimento *et al*., 2000). In the last decade, studies based on extraction of biologically active compounds from plant species used for medicinal purposes are intensively increased (Nascimento *et al*., 2000; Rios and Recios, 2005). Searches for substances with antimicrobial activity in plants are frequent, due to their popular use as remedies for many infectious diseases (Shibato *et al.,* 2005). Plants are rich in different types of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties (Cowan 1999; Lewis 2006). Consequently, the development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants (Erdogrul *et al.,* 2002). Today, it is estimated that relatively 50 % of Western drugs have plant materials (Rodders *et al.,* 1996).

Tokar sha or *sabulun toka* is a traditional medicine that is found mainly in the northwest zone of Nigeria specifically Sokoto, Kebbi and Zamfara. *Tokar sha* is made from millet stalk ash and palm oil which are mixed to make a paste then heat to boil and then cooled to solidify. *Tokar sha* is used in the management of cold, malaria, stomach-ache, abdominal cramp, pile, dyspepsia, epilepsy (Traditional medicinal practitioner). These properties led to the study of its antibacterial property (mainly enteric bacterial).

Bacterial infection has become the most prevalent infectious disease, several semi-synthetic and synthetic drugs are available in practice. However, their effectiveness is affected by their side-effect, contra-indications in children and pregnant women and also rising prevalence of antibiotics resistance of pathogenic microorganisms**.** This spread of antimicrobial resistance in developing countries including Nigeria is associated with complex and interconnected factors, such as excessive and unnecessary prescribing of antimicrobials, increased self-prescribing by people and poor quality of available antimicrobials (Yadesa *et al.,* 2015). This antibiotic resistant pathogenic microorganism raises the demand for finding new alternative antimicrobials agents which have the potential of treating bacterial infections with lesser side effects compared to synthetic drugs.

Although this study is novel, since no research have been done on *tokar sha* but from the Ethnomedicinal survey. It was said to have been used in the treatment of bacterial infection, malaria, cold, epilepsy and related neurologic illness, syphilis, pile, abdominal pain, and cancer, dandruff, and is also used as tooth wash powder, bathing soap and so on. Therefore, this study is aimed at analyzing the phytochemical content and evaluating the antibacterial effect of *tokar sha* using enteric bacterial (enterobacter).

Materials and Methods

Culture organisms

Clinical isolates of two Enteric Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and two Enteric Gram-negative (*Escherichia coli* and *Salmonella* typhi) organisms were used.

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Sample collection

The sample of *tokar sha* was collected from a traditional seller from Kofar Gawo, Central market, Sokoto State, Nigeria in October 2019.

Sample preparation

The collected *tokar sha* was ground to powder using mortar and pestle. A total of 0.04 g, 0.05 g, 0.06 g and 0.07 g was weighed into a small beaker and 2 ml of distilled water was added to each beaker to make and aqueous solution of 20 mg/ml, 25 mg/ml, 30 mg/ml and 35 mg/ml respectively and a glass rod is used to stir the solution until a homogeneous mixture is formed since *Tokar Sha* is completely soluble in water and a foil paper is used to cover the solution to avoid any contamination. Another 0.42 g was weighed and dissolved in 12 mL of distilled water making a solution containing 35 mg/ml and a beaker containing the solution was covered using a foil paper to avoid contamination.

Experimental Animals

Male and female mice weighing between 15 to 25g were purchased from the faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria. The animals were housed in well-ventilated cages in the animal house of the Department of Applied Biology, College of Science and Technology, Kaduna Polytechnic, Kaduna. The mice were allowed to acclimatize for three weeks before the experiment and had access to food and clean water. Principles of laboratory animal care and ethical guidelines for the investigation of experimental pain in conscious animals were observed during experimentation.

Experimental organisms

Aqueous solution of 20 mg/ml, 25 mg/ml, 30 mg/ml and 35 mg/ml of *tokar sha* were tested against a set of four (4) bacteria.

Preparation of inoculums

Inoculum of the isolates was standardized by comparing their turbidity with that of 0.5 McFarland standard.

Phytochemical Screening of *Tokar Sha*

The fraction of the aqueous solution of *Tokar sha* was subjected to phytochemical screening and the following components investigated; Tannins, carbohydrates, flavonoids, Saponins, Cardiac glycosides, Alkaloids, Phytosterols/triterpenoids, oils and Anthraquinones.

Acute Toxicity

Sub-acute oral toxicity test was performed according to the organization of Economic Co-operation and Development (OECD) guideline 407 for testing of chemicals.

The animals were divided into three experimental groups (n=5) animals/group. Three different doses of *Tokar sha* concoction (250,500,1000 mg/kg) were administered orally for 5 consecutive days. All mice were then allowed free access to food and water and were observed for behavioral and physiological variation initially, then continuously for 30min 1 hour. The monitoring of the parameters commenced immediately after administering the extract, for signs of toxicity, which included but were not limited to paw-licking, motor activity, tremors, convulsions, posture, spasticity, opisthotonicity, ataxia, sensations, piloerection, ptosis, lacrimation, exophthalmos, salivation, diarrhoea, writhing, skin colour, respiratory rate and mortality.

Antibacterial Assay of *Tokar Sha*

Screening of *Tokar Sha* for Antibacterial Activity

The Antibacterial activity of the aqueous solution of *tokar sha* were determined using the agar well diffusion method as described by

(Boakye- Yiadom,1979) and (Boyanova *et al.*,2005). Inocula of the isolates were standardized by picking a discrete colony and emulsify it in normal saline to a turbidity equivalent to that of 0.5 McFarland's standard. A 7mm sterile stainless-steel cork borer was used to make wells on the plates. The holes were individually filled with 20 mg/ml, 25 mg/ml, 30 mg/ml and 35 mg/ml of the aqueous solution of *tokar sha* using 2 ml syringe and each well was appropriately labeled. Amoxicillin disc 10 µg was used as a positive control. The plates were left to stand for 2 hours at room temperature to allow diffusion of the solution and were then incubated at 37°C for 24 hours. The assay was done in duplicate for each sample on the four (4) organisms. Results were read by measuring the diameters zone of inhibition (mm) around the wells and the mean value for each was determined.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentrations of aqueous solution of *tokar sha* were determined using the macro broth dilution method as described by (NCCLS, 2000). Nine sets of test tubes each containing sterile broth (5ml) were used. A 70mg/ml stock solution of aqueous solution of *tokar sha* was prepared in distilled water and two- fold serial dilution in the nutrient broth was carried out to give concentrating of 35mg/ml, 17.50 mg/ml, 8.75 mg/ml, 4.38 mg/ml, 2.19 mg/ml, 1.09 mg/ml, 0.55 mg/ml, 0.27 mg/ml and 0.14 mg/ml and 2 test tube are used were one is used as growth control and the other for sterility test. Each set was individually inoculated with a standardized suspension of *Bacillus cereus* and *Escherichia coli* except the one for sterility test. The tubes were incubated at 37°C for 24hours and examined for visible bacterial growth as evidenced by turbidity. The lowest concentration that not supports growth was taken as the minimum inhibitory concentration.

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Determination of Minimum Bactericidal Concentration

The minimum Bactericidal concentration of an aqueous solution of *tokar sha* on the two bacteria species was determined by sub-culturing the test dilution from the minimum inhibitory concentration where no visible growth was seen as described by (NCCLS, 2000). A sterile inoculating loop was used to streak the different test dilutions onto the fresh nutrient agar previously prepared. Each test was marked into the quadrant and a set of individual dilutions of the solution on the different organisms were streaked onto separate plates giving a total of two plates. The plates were incubated upside down at 37°C for 34 hours. The minimum Bacterial concentration was determined by where there was no visible growth on the plates.

Results Presentation

Phytochemical Screening of *Tokar Sha*

The result of phytochemical screening of Tokar Sha obtained shows the presence of some primary and secondary metabolites including; carbohydrates, oils, cardiac glycosides, saponins and phytosterols as presented in table 1.

Table 1: Result of Phytochemical Screening of *Tokar sha*

Constituents	Results			
Alkaloids	-			
Carbohydrate	+			
Cardiac glycosides	+			
Tannins	-			
Saponins	+			
Phytosterols/triterpenoids	+			
Flavonoids	-			
Oils	+			
Anthraquinones	-			

Keys: - = Negative, + = Positive

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Acute Toxicity Studies of *Tokar Sha* Using Limit Dose Test

No death was recorded in the mice orally administered with 5000mg doses of *Tokar sha.* The concoction was well tolerated by the mice without any overt signs of toxicity. The result is shown in table 2.

Table 2: Result of Oral acute toxicity studies of *Tokar sha* in Mice

Dose (mg/kg)	Number of Animals Used	%Mortality
5000	0/1	0
5000	0/1	0
5000	0/1	0
5000	0/1	0
5000	0/1	0

The LD50 was calculated to be > 5000mg/kg

Antibacterial Activity of *Tokar Sha*

The results of Antibacterial screening revealed that the aqueous solution of *tokar sha* has activity on *Escherichia coli* and *Bacillus cereus* but it has no activity on *Staphylococcus aureus* and *Salmonella* typhi. The zone of inhibition ranges from 12mm to 25mm with *Escherichia coli* having the highest zone of inhibition of 25mm and *Bacillus cereus* with 12mm as the lowest as shown in tables 3 and 4 respectively.

Table 3: Antibacterial activity of Tokar Sha on *S. aureus, B. cereus, E. coli* and *S.* typhi

	Mean diameter zone of inhibition (mm)			
ORGANISMS	*S. aureus*	*B. cereus*	*E. coli*	*S.* typhi
CONCENTRATION (mg/ml)				
35	-	20	25	-
30	-	14	23	-
25	-	12	23	-

20	-	-	21	-

KEY: - = No zone of inhibition

Table 4: Diameter zone of inhibition of Standard Antibiotics (Amoxicillin) on *S. aureus, B. cereus, E. coli* and *S.* typhi

	Mean diameter zone of inhibition (mm)			
Organisms	*S. aureus*	*B. cereus*	*E. coli*	*S.* typhi
Concentration (µg/ml)				
10	36	42	10	15

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) against the two organisms that are susceptible to *Tokar sha* was determined and found to be 35 mg/ml being the lowest concentration for both *Escherichia coli* and *Bacillus cereus* whereby, the remaining concentration ranges from 17.5 mg/ml to 0.14 mg/ml has the growth of organisms as shown in the table 5.

Table 5: Minimum Inhibitory Concentration of *tokar sha* on *B. cereus* and *E. coli*

ORGANISMS	*B. cereus*	*E. coli*
Aqueous solution of Tokar Sha (mg/ml)	35	35

Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) results (Table 6) shows that the aqueous solution of *tokar sha* has the lowest MBC at 35mg/ml for both *Escherichia coli* and *Bacillus cereus* whereas the remaining concentration ranges from 17.5 mg/ml to 0.14 mg/ml there is no inhibition of Bacterial growth.

Discussions

The phytochemical screening results obtained show that *tokar sha* contains a variety of primary and secondary metabolites namely; carbohydrate, cardiac glycosides, phytosterols, saponins, and oils.

Which contribute greatly to its diverse activity including antibacterial activity.

In screening natural products for pharmacological activity, assessment and evaluation of the toxic characteristics of a natural product extract, fraction, or compound are usually initial steps taken. In this study, *Tokar sha* at doses up to 5000 mg/kg had no treatment-related signs of toxicity or mortality in any of the animals tested during 24 hours of observation. The LD50 of *Tokar sha* was estimated to be more than 5000 mg/kg. Therefore, *Tokar sha* can be categorized as highly safe extracts since substances possessing LD50 higher than 5000 mg/kg body weight are non-toxic (Buck *et al.,* 1976).

The Antibacterial screening results show that *tokar sha* has activity on two of the test organisms (*Escherichia coli* and *Bacillus cereus*) while the other two organisms (*Staphylococcus aureus* and *Salmonella* typhi) are found resistant. The highest zone of inhibition been 25 mm and 20 mm for *E. coli* and *B. cereus* at 35 mg/ml and the lowest zone of inhibition 12 mm at 25 mg/ml for *Bacillus cereus, tokar sha* shows better Antibacterial activity on *E. coli* than *B. cereus,* because *B. cereus* has no zone of inhibition at 20 mg/ml concentration whereas *E. coli* has 21 mm zone of inhibition. Thus, it is seen that the zone of inhibition in each organism increases with an increase in concentration making the antibacterial effect to be dose-dependent. Antibacterial activity seen can be because the cell walls of *B. cereus* which is a gram-positive bacterium are made up of main peptidoglycan. Peptidoglycan is found to be distorted by long-chain fatty acids that are found in palm oil an active ingredient in *tokar sha*. The activity against *B. cereus* and *E. coli* therefore, could be attributable to the palm oil present in the *tokar sha* (Ugbogu, 2006). Ugbogu, (2006) reported that palm oil has an inhibitory effect on *B. cereus* and *E. coli.* The major fatty acids in palm oil used for the production of *tokar sha* are lauric acid, myristic acid and oleic acid. Certain fatty acids (medium-chain saturates) and their derivatives have adverse effects on various microorganisms (Kabara, 1978).

Tokar sha also contain saponins as already seen in the phytochemical analysis result and since saponin are known to be a detergent-like substance having Antibacterial activity which disturbs the permeability

of the bacterial outer membrane by interacting with lipid. A part of Proteus lipopolysaccharide LPS that is about 90 % of the surface of naturally cholesterol - free gram-negative bacteria cell-wall (Arabski *et al*., 2009). There was no observed inhibitory effect on *S. aureus* and *S. typhi* by *tokar sha* at all concentrations used, this resistance can be said to be due to chromosomal mutation that lower the permeability of the agent or acquisition of resistant (R) plasmids and transposons (Arora, 2004).

The MIC and MBC results of both susceptible organisms (*Escherichia coli* and *Bacillus cereus*) were found at a concentration of 35 mg/ml. Although there was a record of the zone of inhibition at lower concentrations (20 mg/ml and 25 mg/ml) than this from antibacterial screening. Thus, because the MBC value was in all cases the same as in MIC value, it can be deduced that *tokar sha* shows only bactericidal activity with no bacteriostatic activity.

Conclusions

In the present study, it was revealed that *tokar sha* exhibited antibacterial activity against the test organisms *Escherichia coli* and *Bacillus cereus* providing a support on its ethnomedicinal uses in treating piles, stomach cramps and other entero-infections.

It is recommended that further studies should be conducted on *tokar sha* to exploit and confirm other ethnomedicinal uses that had been attributed to *tokar sha* so that it can be produced industrially employing hygienic standards.

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