Inhibitory activity of bark and fruit extracts from *Tetrapleura teraptera* (Schumach. and Thonn) Taub against methicillin resistant *Staphylococcus aureus* (MRSA)

Oluseyi Fatile, Abraham O. Nkumah, Omonike O. Ogbole

Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria

Corresponding author: Omonike O. Ogbole
Email: nikeoa@yahoo.com; Phone no: +2348056434577

ABSTRACT

Background: *Tetrapleura tetraptera* an indigenous African medicinal plant commonly known as Aridan fruit, has been reportedly used for traditionally treatments of various ailments including infectious diseases.

Objective: This study was designed to investigate the anti-methicillin resistant *Staphylococcus aureus* activity of *Tetrapleura teraptera* bark and fruit.

Methods: Pulverized parts were extracted into absolute methanol at room temperature and concentrated using rotary evaporator. Using agar well diffusion and agar dilution method, sensitivity test and minimum inhibition concentration (MIC) were determined against six strains of MRSA. Data were analyzed using Microsoft excel.

Results: Methanol extracts of bark and fruit elicit inhibitory properties on the six strains of MRSA. At 200 mg/mL, the bark showed most significant inhibitory activity against SA422 and with zone of inhibition (ZOI) of 27.65±0.35 mm compared to gentamycin of 17.95±0.05 mm, it also demonstrated significant inhibitory activities at different concentration on the other MRSA strains. At 200 mg/mL, the fruit extract was very active as compared with the standard drug, especially on SA422 where it showed similar and comparable (ZOI) of 19.5±0.50mm with gentamycin. The bark extract had the best minimum inhibition concentration (MIC) of 0.78 mg/mL for SA422, SA776, and SA423 while the fruit also had MIC of 0.78 mg/mL on SA422 and 423.

Conclusion: Methanolic extract of the bark showed great inhibitory activity against tested organism even at the lowest concentration, justifying its wide use in traditional management of infectious diseases.

Keywords: Anti-microbial, *Staphlococcus aureus*, Mimosaceae, *Tetrapleura tetraptera*, aridan

Activité inhibitrice de l'extrait d'écorce et de fruit de *Tetrapleura Teraptera* (Schumach. et Thonn) Taub contre le *Staphylococcus aureus* résistant à la méticilline (SARM)

Oluseyi Fatile, Abraham O. Nkumah, Omonike O. Ogbole

Département de pharmacognosie, Faculté de pharmacie, Université d'Ibadan, Ibadan, Nigeria

Auteur correspondant: Omonike O. Ogbole
Email: nikeoa@yahoo.com; Téléphone : +2348056434577

RÉSUMÉ

Contexte : *Tetrapleura tetraptera,* une plante médicinale africaine indigène communément connue sous le nom de fruit d'Aridan, aurait été utilisée traditionnellement pour le traitement de diverses affections, y compris les maladies infectieuses.

Objectif : Cette étude a été conçue pour examiner l'activité de l'écorce et du fruit de Tetrapleura teraptera contre le Staphylococcus aureus résistant à la méticilline.

Méthodes : Les parties pulvérisées ont été extraites dans du méthanol absolu à température ambiante et concentrées à l'aide d'un évaporateur rotatif. En utilisant la méthode de diffusion en gélose et la méthode de dilution en gélose, le test de sensibilité et la concentration minimale d'inhibition (CMI) ont été déterminés contre six souches de SARM. Les données ont été analysées à l'aide de Microsoft Excel.

Résultats : Les extraits méthanoïques de l'écorce et du fruit induisent des propriétés inhibitrices sur les six souches de SARM. À 200 mg/mL, l'écorce a montré l'activité inhibitrice la plus significative contre SA422 et avec une zone d'inhibition (ZOI) de 27,65±0,35 mm par rapport à la gentamycine de 17,95±0,05 mm, elle a également démontré des activités inhibitrices significatives à différentes concentrations sur les autres souches de SARM. À 200 mg/mL, l'extrait de fruit était très actif par rapport au médicament standard, en particulier sur SA422 où il a montré une activité similaire et comparable (ZOI) de 19,5±0,50 mm avec la gentamycine. L'extrait d'écorce avait la meilleure concentration minimale d'inhibition (CMI) de 0,78 mg/mL pour SA422, SA776, et SA423 tandis que le fruit avait également une CMI de 0,78 mg/mL sur SA422 et 423.

Conclusion : L'extrait méthanoïque de l'écorce a montré une grande activité inhibitrice contre l'organisme testé même à la concentration la plus faible, ce qui justifie sa large utilisation dans la gestion traditionnelle des maladies infectieuses.

Mots clés : Antimicrobien, *Staphylococcus aureus*, Mimosacées, *Tetrapleura tetraptera*, aridan

Introduction

Medicinal plants are a rich source of bioactive substances, and have been shown as a good source material for sourcing new drugs.[1] They form the basis of many modern pharmaceuticals.[2,3] Plant based traditional medicine system continues to play a crucial role in health care, with about 80% of the world's inhabitants relying on traditional medicines for their primary health care.[4]

Infectious diseases are one of the most important challenges in human health due to their high incidence and outbreak rate. Globally, they are difficult to control because of antimicrobial resistance, this problem is now worsened by the emergence of multidrug resistance strains.[5] Current reports have indicated that *Staphylococcus aureus* is not only multidrug resistant pathogens,[6] but also a broadly drug-resistant and pan drug-resistant bacteria. It has been reported worldwide that *S. aureus* isolates are increasingly resistant to a greater number of antimicrobial.[7-9] *Staphylococcus aureus* also referred to as the golden staph is also responsible for the majority of nosocomial infections. It is a facultative anaerobic gram positive *coccal bacterium*. It is frequently part of the skin flora found in the nose and on the skin and tis manner about 20% of the human population are long term carrier of *S. aureus* Microbes are closely associated with the health and welfare of human being.[10] The failure of chemotherapeutics and antibiotics resistance created by pathogens has opened the gate towards the utilization of medicinal plants as antimicrobial agents.[11]

Plants are used in different regions of the world for the treatment of microbial diseases. Plants with phenolic compounds are commonly known for their antioxidants, anti-inflammatory and antimicrobial activities.[12] Traditionally, *Tetrapleura teraptera* as food and medicine is a widely use plant among the local herbs sellers and healing homes, in southwestern Nigeria for the treatment of skin infection caused by *S. aureus*. It has also been used to treat a lot of diseases like leprosy, female reproductive issues, stomach ailments, fever, malaria, convulsions, flatulence and gonorrhea.[13] It is used by men for birth control and used in pepper soup to aid in cleansing the womb after child birth. *Tetrapleura teraptera* belong to the family Fabaceae - Mimosoideae and it is locally called by the Yoruba people in Nigeria "aidan tree" and by the Uganda people has "munyegenye" *T. tetraptera* is common on the fringe of the West African rainforest belt. Trees are widespread in tropical Africa, in forest, especially secondary forest, and they are at their best in the rainforest. The species is found throughout the high forest zone, in riverian forest, in the southern savannah-woodland and in the forest outliers in the African plains.[14-17]

Antimicrobial agents of plant origin are not associated with many side effects and have an enormous therapeutics potential to heal many infectious disease.[18] Therefore, the research is driven by the need to search for new antimicrobial molecule of plant origin without the carcinogenic effect of synthetic antimicrobials and the microbial resistance to their potency.

MATERIALS AND METHODS

Plant material collection, identification and authentication.

Tetrapleura teraptera plant (fruit and bark) were purchased from herb sellers at Ojee market, Ibadan, Oyo State, Nigeria, in the month of August 2016. Plant specimen were collected, identified and authenticated at Forest Herbarium Ibadan (FHI), with voucher no: 110127. Herbarium specimen were prepared using standard procedure. Voucher specimen were deposited at FHI and at the Department of Pharmacognosy Herbarium, University of Ibadan (DPHUI)

Sample preparation and extraction

The fruits and barks were air dried for a three (3) weeks. 500 g of each of the part was pulverized using a mechanical grinder. Samples were extracted into methanol by cold maceration for 72 hours. The crude methanol extracts were concentrated using a rotary evaporator. The extract was then refrigerated at 4^{o}C until needed.

Test microorganism

Bacterial strains

Three of the *S. aureus* strains (Sa422, SA776, and SA45) used in the current study were confirmed clinical isolates at the Department of Pharmaceutical Microbiology, University of Ibadan and have been phenotypically identified as MRSA solely through resistance to cefoxitin (10 µg/mL). While the remaining three (SA264, SA423, SA277) were identified by both phenotypic and genotypic (detection of mecA gene by PCR) methods as reported earlier[19,20.] All the bacterial strains were grown and maintained on nutrient agar slants.

Antimicrobial activity screening

Antimicrobial sensitivity test

Agar well diffusion method was used in determining antimicrobial activities of the extracts, 8.4 g of nutrient agar was dissolved in 300 mL of water and was placed in a water bath at 100°C for thirty minutes after which they were stabilized at 45°C for 15 minutes. Bacterial cell suspensions adjusted to 0.5 McFarland standards (108 CFU/ml) were prepared from overnight culture of the test organism. One hundred microliter (100µL) of bacteria culture was seeded into 15 mL of melted and cooled *Muller Hinton agar* (MHA) (Oxoid, UK) and poured into sterile Petri-dishes after mixing thoroughly; the agar was then allowed to set. Each molten and cooled *Muller Hinton agar* (18 mL) (Oxoid, UK) was inoculated with 0.2 mL of overnight broth culture of the test organisms. The seeded agar was poured into separate sterile petri-dishes and allowed to set. Using a sterile cork borer (8 mm), six equidistance wells were bored into the set agar. The extracts were reconstituted by dissolving 1 g of each in 5 mL (200 mg/mL) of methanol, this stock was serially diluted to obtain a range of concentration from 200 - 0.78 mg/mL. The wells were filled with 100µL of varying concentration of each extract from the plant part. Control wells were filled with 10mg/mL of gentamycin. The plates were allowed to stand for one hour to allow adequate diffusion of the extracts and the drug control. The plates were then incubated at 37^{o}C for 24 h. All experiments were done in triplicate. Zones of inhibition were measured in millimeter (mm) and the average determined Sidqui *et al*.[21] The antimicrobial activity was expressed as the mean zones of inhibition diameters (mm) produced by the extracts.

Determination of minimum inhibitory concentration (MIC)

Nutrient agar (11.2 g) was dissolved in 400 mL of sterile distilled water and homogenized in a water bath for 20 minutes. Then 18 mL of this was dispensed into each universal bottle and autoclaved. Nine concentrations of each extract were prepared and 2mL of each concentration of the extracts were seeded into 18mL of molten nutrient agar aseptically. 0.65 g of nutrient broth was dissolved in 50 mL of distilled water and 5 mL was dispensed into test tubes and autoclaved. The organisms were inoculated into the autoclaved broth. The organisms were diluted by adding 0.1 mL of each organism into 9.9mL of sterile water. They were then streaked on the plates using sterilized swab stick. Plates were incubated for 24hrs at 37^{o}C. The MIC is read as the lowest concentration of extracts at which there is no visible growth of the microorganism.

RESULTS

The bark extract of *T. tetrapleura* had significant activity against different strains of MRSA at all concentration (with activity up to the lowest concentration of 0.78 mg/mL). At 200 mg/mL, the activity of the bark extract against SA422 (ZOI) of 27.65±0.35 mm was better than that of gentamycin (17.95±0.05). Other strains were equally susceptible to the bark extract with activities similar to that of standard drug, (Table 1).

At 200 mg/mL (Table 2), the fruit extract showed comparable inhibitory activity on MRSA strains (which is very obvious) especially with SA423 where it has similar zone of inhibition (ZOI of 19.5±0.50mm) as gentamycin.

Table 3 shows the minimum inhibition concentration (MIC) of both extracts. The bark extracts have MIC of 1.56 mg/mL on strains SA246, SA277 AND SA45, MIC of 0.78 mg/mL on SA 422, 776 and MIC 0.78 mg/mL on SA423. The strains were not as sensitive to the fruit extract with MIC of 6.25 mg/mL on SA45; 3.12 mg/mL on SA277, SA776; 1.56 mg/mL on SA422 and the most sensitive is SA246 with MIC of 0.78 mg/mL.

Table 1: Inhibitory activity of the bark extract of *Tetrapleura teraptera*

Conc. mg/mL	Zone of inhibition (ZOI) (mm)					
	SA422	**SA264**	**SA423**	**SA277**	**SA776**	**SA45**
200	27.65±0.35	24.75±0.25	27.65±0.35	21.75±0.25	23.75±0.25	20.50±0.50
100	24.75±0.25	23.85±0.85	24.75±0.25	21.45±0.45	18.25±0.25	20.25±0.25
50	24.10±0.10	22.50±0.50	24.00±1.00	18.30±0.30	16.25±0.25	16.50±0.50
25	20.25±0.25	18.75±0.75	23.10±0.10	12.15±0.15	17.75±0.25	15.50±0.50
12.5	14.30±0.30	12.95±0.05	20.25±0.25	11.75±0.25	11.80±0.20	9.50±0.50
6.25	14.30±0.30	4.25±0.25	14.30±0.30	5.60±0.40	5.85±0.15	4.75±0.25
3.12	12.20±0.20	2.50±0.50	12.20±0.20	3.25±0.25	5.25±0.25	2.05±0.50
1.56	7.400±0.40	0.50±0.50	7.40±0.40	0.75±0.25	2.50±0.50	0.00±0.00
0.78	3.50±0.50	0.00±0.00	3.50±0.50	0.00±0.00	0.00±0.00	0.00±0.00
Gent. (100 µg/mL)	17.95±0.05	27.65±0.35	27.95±0.05	26.75±0.25	30.50±0.50	22.75±0.25

Table 2: Inhibitory activity of the fruit extract of *Tetrapleura teraptera*

Conc. (mg/mL)	Zone of inhibition (ZOI) (mm)					
	SA422	**SA264**	**SA423**	**SA277**	**SA776**	**SA45**
200	18.15±0.15	15.75±0.25	19.50±0.50	14.10±0.10	11.60±0.40	10.75±0.25
100	16.21±0.10	14.25±0.25	17.75±0.25	10.25±0.25	9.75±0.25	9.25±0.25
50	14.10±0.10	12.75±0.25	16.25±0.25	8.25±0.25	6.85±0.15	6.75±0.25
25	12.15±0.15	11.75±0.25	11.75±0.25	5.15±0.15	5.75±0.25	2.25±0.25
12.5	11.60±0.40	11.60±0.40	9.75±0.25	3.10±0.10	4.15±0.15	0.00±0.00
6.25	4.90±0.10	6.75±0.25	9.90±0.10	0.75±0.25	2.25±0.25	0.00±0.00
3.12	2.05±0.50	4.10±0.10	17.75±0.25	0.00±0.00	0.00±0.00	0.00±0.00
1.56	0.00±0.00	0.95±0.05	15.25±0.25	0.00±0.00	0.00±0.00	0.00±0.00
0.78	0.00±0.00	0.75±0.25	9.75±0.25	0.00±0.00	0.00±0.00	0.00±0.00
Gent. (100 µg/mL)	19.75±0.25	15.75±0.25	19.5±0.50	15.75±0.25	16.75±0.25	19.25±0.25

Table 3: Minimum inhibition concentration result for the bark and fruit extract of *Tetrapleura teraptera*

Organisms	Minimum inhibition concentration mg/mL	
	Bark	**Fruit**
SA422	0.78	1.56
SA246	1.56	0.78
SA423	0.78	0.78
SA277	1.56	3.12
SA776	0.78	3.12
SA45	1.56	6.25

DISCUSSION

Plant materials over several decades have been recognized to exhibit antimicrobial properties. The use of these plant materials as preservatives and as means of preventing microorganism development in foods has become the subject of extensive studies.[22] This plant based traditional medicine system continues to play a crucial role in health care, with about 80% of the world's inhabitants relying on traditional medicines for their primary health care.[23]

Tetrapleura tetraptera, family Mimosaceae, has widely varied applications in Nigerian folk medicine. The pods notably have an appealing culinary use. Apparently, they are used to prepare soups for mothers from the first day of delivery to relieve post parturition contraction and as a lactation aid.[24] At the same time most of the folkloric claims agree in the traditional use of the fruit for management of convulsion, leprosy, inflammation and rheumatoid pains.[25] The antimicrobial activity of this plant has been exploited in the formulation of herbal soap bases using palm kernel oil.[26]

The purpose of this study was to examine the antibacterial effects of *T. tetraptera* extract on different strain of Methicillin resistant *Staphylococcus aureus* and also determine what concentration show reasonable level of inhibition to the pathogenic bacteria. Such studies are essential if the full potential of *T. tetraptera* to treat skin infection is to be exploited.

The strains are more susceptible to back extract at MIC of 1.56 mg/mL as compared to that of the fruit extract. This implies that while both the fruit and bark extract were very active, greater activity resides more in the bark extracts. The results above have established the fact that *T. tetraptera*, both bark and fruits, contain anti MRSA constituents, and justified its great utilization among local herb sellers in particular, it has been reported to be vital to rural sustainability.[13,27] The powdered dried bark is used to formulat several concoction and decoction in the treatment of various illnesses, especially skin infections [27-29] and its antimicrobial properties against multiple microorganism has been severally reported.[15,30,31] It has been documented for traditional treatment of wound and wound infection[32-34] Antibiotic resistant organisms, such as methicillin resistant *Staphylococcus aureus* (MRSA), are more commonly encountered in slow healing wounds.[35] It is the capacity for wound re-epithelization and significant anti MRSA activity that makes it a good candidate for the treatment of slow healing wounds, especially in the case of heavily infected wounds and immunocompromised patients.

This study is restricted to several strains of MRSA. Other gram positive and gram-negative infectious organisms should be tested to determine broad spectrum activity of the extracts. Compounds/phyto-constituents responsible for the activities in the extract should be determined. This is therefore, suggested that further work on isolation and characterization of the compounds responsible for the activities be carried out.

CONCLUSION

This study confirms that extracts from *Tetrapleura tetraptera* has antimicrobial properties against methicillin resistant *Staphylococcus aureus* and justifies its wide use among the herb sellers in Ibadan, Nigeria.

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