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In-Vitro Evaluation of The Anti-Bacterial Effect of Gossypium Barbadense Extracts on Isolates of Salmonella Typhi

Iyevhobu, K.O.[1,2*], Edo, E.O.[3], Airefetalor, A.I.,[1,2], Jabbo, A.A.[3], Dare, D.I.[4], Ken-Iyevhobu, B.A.[5], Oseni, D.I.[5]

[1]*Department of Public Health, National Open University of Nigeria, Uromi Community Study Centre, Uromi, Edo State, Nigeria.*
[2]*Lassa Fever Enable Study CEPI/ISTH Irrua, Edo State, Nigeria.*
[3]*National Ear Care Centre, Kaduna, Kaduna State, Nigeria*
[4]*Department of Medical Microbiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria.*
[5]*St. Kenny Research Consult, Ekpoma, Edo State, Nigeria.*

Corresponding Author:* Iyevhobu, K.O. Department of Public Health, National Open University of Nigeria, Uromi Community Study Centre, Uromi, Edo State, Nigeria. 2. Lassa Fever Enable Study CEPI/ISTH Irrua, Edo State, Nigeria.

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Abstract

Gossypium, commonly known as “cotton plant” is well known for its versatile significance in traditional medicine. Salmonella is a genus of gram-negative, motile bacilli belonging to the family Enterobacteriaceae. The study was aimed at evaluating the antimicrobial potential of leaf extracts of Gossypium barbadense against Salmonella typhi isolates. Extracts at varying dilution concentrations were prepared from the infusion of Gossypium barbadense leaves (with stalk) in three different aqueous extraction solvents (7up, distilled water and ethanol). The plant extracts were studied for their antimicrobial activities against a clinical isolate and Salmonella typhi.7up extract showed resistance at all the dilution concentrations to both isolates. From the activity of the distilled water extract in this study, Gossypium barbadense showed innate anti-salmonella properties and it was observed that distilled water seemed to be a better vehicle for the extraction of the anti-salmonella properties of Gossypium barbadense leaves than 7up.

Keywords: *in-vitro, anti-bacterial, gossypium barbadense, medicinal, salmonella typhi, antibiotics, typhoid*

Introduction

Salmonella is a genus of gram-negative, motile bacilli with peritrichous flagella belonging to the family Enterobacteriaceae (Crump & Mintz 2010). Salmonella is responsible for infections that affects both humans and animals. There are two species of Salmonella; Salmonella enterica and Salmonella bongori (Eng et al., 2015). Salmonella enterica causes infections in warm-blooded animals and has six species with over 2500 serotypes (Ralph, 1996). Typhoidal salmonellosis is caused by the following serotypes of Salmonella enterica: Salmonella typhi, which is the causative agent of typhoid fever; and Salmonella paratyphi A, B and C, which cause paratyphoid fever (CDC, 2011).

Medicinal plants have been demonstrated to play an important role in African pharmacopoeia (Osanaiye et al., 2018). However, scientific investigations and information on the therapeutic potential of these medicinal plants are limited (El-Mahmood et al., 2010). Plants have been noted to be one of the most important sources of active substances with therapeutic potential to resolve a variety of diseases in humans (Adekola et al., 2015). Many scientific studies have therefore been undertaken to identify and determine the efficacy of the various plants used in traditional remedies, gossypium is one of such plants. Gossypium, commonly known as “cotton plant” is the most economically important member from the Malvaceae family and is well known for its versatile significance in the production of textile, livestock feed and traditional medicine (Egbuta et al., 2017). There are four commercially important species of Gossypium, Gossypium hirsutum L., Gossypium herbaceum L., Gossypium arboretum L. and Gossypium barbadense L. (Ade-Ademilua & Okpoma, 2018; Khaleequr et al., 2012). Gossypium hirsutum is the most widely cultivated gossypium

specie throughout the warmer parts of the world and has become the main cotton of commerce, attributing to about 90% of the world's cotton (Al-Snafi, 2018; Jerkins, 2003). The various plant parts of these species were mentioned to have been used by ancient people in folklore medicine to cure or manage a variety of illnesses like bronchitis, asthma, diuresis, diarrhea and stomach-ache amongst others (Al-Snafi, 2018). Many rural communities in Nigeria use crude Gossypium extracts as their go-to alternative remedy for typhoid. The extracts are used alone or alongside conventional drugs or other plants extracts. In Okpameri, a rural community in Akoko-Edo Local Government Area, Nigeria, these crudes are made using different extraction solvents, commercially available 7up drinks being the most common.

Conventional antibiotic treatment and drug therapy have been set up to treat typhoid fever featuring antibiotics like ciprofloxacin, azithromycin, ceftriaxone and chloramphenicol (Uttam & Bandyopadhyay, 2017). Treatment routines are to be adjusted when culture and sensitivity test results are available (CDC, 2011). However, there are cases where treatment routines using these conventional drugs fail or seem ineffective and symptoms persist. When such situations arise, many communities in Nigeria fall back to the use of homemade traditional remedies from local plants such as Gossypium. The use of traditional medicine was and still is a common practice by many cultures of the world. In Nigeria, the use of traditional remedy is practiced by a large proportion of the population especially in rural communities with insufficient or inaccessible modern health care facilities and low-income earners who can't afford the facilities available (El-Mahmood et al., 2010; Kudi et al., 1998). About 80% of the rural population and some urban dwellers depend to a large extent on traditional medicine only turning to modern conventional therapies when disease symptoms advance or become unmanageable (El-Mahmood et al., 2010).

The emergence of multi-drug resistant (MDR) strains of Salmonella typhi poses a challenge to the treatment of infected patients and a lack of effective drug therapy may lead to an increase in morbidity and mortality rates (Eng et al., 2015). In addition, there is an increasing demand for safer and more affordable organic (natural) health routes (Osanaiye et al., 2018). Consequently, these issues have resulted in scientific studies being undertaken to either seek out new alternatives or rediscover and develop traditional plant remedies. The study was aimed at evaluating the antimicrobial potential of leaf extracts of Gossypium barbadense against Salmonella typhi isolates.

Materials and Methodology

Collection of Materials

Ethnomedical Information

Ethnomedical information which formed part of the basis of this study was gotten through interviews and open-ended conversations with some elderly natives of Okpameri community and their young counterparts who were known to dabble in traditional medicine. According to these natives, the traditional extract was made by chopping up the leaves and placing them in a container. 7up (made by Pepsi corperation) or Sprite drink (made by Coca-cola) was then added to the diced-up leaves in enough quantity to suspend them. The preparation was then allowed to sit for a minimum of 12 hours (many preferred to let it sit overnight), after which it is agitated, filtered and drunk. Sometimes, a fresh volume of 7up is added to the filtered residue and allowed to sit for another 12 hours after which it is taken as evening dose before bedtime. A new extract is prepared every day in readiness for when it is to be taken. This preparation is to be drunk consistently, day and night for three days.

Collection of Test Organisms

Clinical and standard strain isolates of Salmonella typhi were obtained from the Microbiology Laboratory of the University of Ibadan, Oyo State, Nigeria. The clinical isolate was obtained from human stool culture.

Collection of Plant Material

Members of the genus Gosssypium L. are generally used indiscriminately in traditional medicine without recognition or credence to the fact that there are different species which are likely to have different medicinal capabilities (Ade-Ademilua & Okpoma, 2018). Gossypium barbadense was chosen based on its reported predominance in most parts of Nigeria (Ademilua et al., 2017). Gossypium barbadense leaves were collected from garden Gossypium barbadense plants in Okpameri, Akoko-Edo L.G.A., Edo-State, Nigeria.

Extraction Method

Solvent extraction is a common extraction principle used when extracting natural components for crude/traditional medicines, the others include distillation, sublimation and pressing. The conventional extraction methods are maceration, percolation and reflux extraction but the downside is

that large volume of solvent is usually required unlike some modern methods such as Supercritical Fluid Extraction (SFE), Pressurized Liquid Extraction (PLE) and Microwave Assisted Extraction (MAE) (Zhang et al., 2018). The extraction method used in this study was cold infusion. The preference for this method is based on its use by the Okpameri people to obtain their crude extracts.

Extraction Procedure

Fresh infusions were made by macerating the plant material then suspending in the different solvents for 24 hours. The solvent to sample ratio used was 25:1. The fresh leaves were washed under clean running water then blotted dry using sterile filter paper. 20g of the fresh leaves was aseptically cut into small pieces and placed in a sterile container and 500ml of solvent was added to suspend the macerated leaves. The preparation was allowed to sit for 24 hours at room temperature and the infused extract was then separated from the macerated residue by filtration. Serial double dilution (1 in 2) was then carried out to get the following dilution concentrations: 100, 50, 25 and 12.5 (Abubakar & Haque, 2020).

Extract Characteristics

The following was observed about the unfiltered extracts after 24 hours of infusion; the 7up extract took on a purplish-red (grape-wine like) hue, the ethanol extract took on a bright green colour while the distilled water extract had a light brown appearance.

Laboratory Analysis

The method used for determining the anti-salmonella activity of the prepared Gossypium extracts was agar well diffusion method using Mueller-Hinton agar. The media was prepared in accordance to the manufacturer's standard (38g in 1000ml of distilled water). Amoxicillin-clavulanate and gentamicin, both in 30mg concentrations, were used as positive reference standards (controls) for the two isolated strains of Salmonella.

Procedure

The Mueller-Hinton test agar plates were aseptically prepared and allowed to solidify, a colony of the test organism was emulsified and suspended in 3ml of sterile normal saline using a sterile wire loop. The test organisms were introduced and evenly spread over the gelled entire agar surface using sterile swab sticks. The inoculated plate was left at room temp for 30minutes to allow for proper seeding of the medium. Wells with diameter of 6mm were aseptically punched into the seeded agar with a sterile standard cork-borer, a sterile syringe was used to introduce each antimicrobial agent or extract solution at desired concentration into the wells. The agar plates were then incubated aerobically at 37° C for 24 hours. Gentamicin(30mg) and Amoxicillin-Clavulanate(30mg) were used as positive controls. The diameters of the zones of inhibition were measured and recorded to the nearest millimeter (mm) (Mounyr et al., 2016).

Data Analysis

Analysis was carried out in duplicates for consistency. The data obtained from the reaction of the two isolates to the extracts were calculated and reported as mean. The test results and the positive control were compared. The statistical significance was calculated using the Interactive Chi Square software. $P > 0.05$ is considered not significant, $P < 0>$

Results

A total of 12 extracts at varying concentrations (100mm, 50mm, 25mm and 12.5mm) were prepared from the infusion of Gossypium barbadense leaves (with stalk) in three different aqueous extraction solvents. The plant extracts were studied for their antimicrobial activities against a clinical isolate and a typed isolate (ST ATCC 14028) of Salmonella typhi, results of which are shown in table 1 and 2. Growth inhibition was determined by measuring the diameter of inhibition surrounding the diffusion well. The relative inhibition was calculated for each extract and recorded as a mean of duplicates.

Table 1 shows the activity of the various extracts (7up, distilled water and 100% ethanol) against clinical isolate of Salmonella typhi. A collective total of 7 extracts prepared from distilled water and ethanol solvents at different dilution concentrations (100, 50, 25, 12.5) showed activity against the clinical isolate. 7up extract showed all-round resistance at all the dilution concentrations. Distilled water extract showed activity at dilution concentrations of 100 (27mm), 50 (26mm), 25 (24.2mm) and 12.5 (20mm). Ethanol extract showed activity at dilution concentrations of 100 (10.5mm), 50 (9.5mm) and 25 (8.5mm). Activity was measured and reported as diameter of inhibition zones (mm). Insignificant or no activity was reported as R (resistant).

Table 2 shows the activity of the various extracts (7up, distilled water and 100% ethanol) against typed isolate of Salmonella typhi (ST ATCC 14028). A collective total of 5 extracts prepared from distilled water and ethanol solvents at different dilution concentrations (100, 50, 25, 12.5) showed activity against the typed isolate. Similar to table 1, 7up extract also showed all-round resistance at every

dilution concentration. Distilled water extract showed activity at dilution concentrations of 100 (24mm), 50 (20mm), 25 (18.5mm) and 12.5 (14mm). Ethanol extract only showed activity at dilution concentration of 100 (10.5mm). Activity was measured and reported as diameter of inhibition zones (mm). Insignificant or no activity was reported as R (resistant).

Table 3 shows the activity of amoxicillin-clavulanate and gentamicin (which served as positive controls) against the clinical and typed isolates. Activity measured as diameter of inhibition zones was 26mm for amoxicillin-clavulanate and 25mm for gentamicin against the clinical isolate while against the typed isolate was 22mm for amoxicillin-clavulanate and 26mm for gentamicin.

Table 1: Mean diameter of inhibition zones at different extract concentrations against the clinical isolate

Extraction solvents	Dilution concentration					P value
	100 (mm)	***50 (mm)***	***25 (mm)***	***12.5 (mm)***	***0 (pure solvent)***	
7up	*R*	*R*	*R*	*R*	*R*	
Distilled water	*27*	*26*	*24.2*	*20*	*R*	*Pc = 0.030*
100% Ethanol	*10.5*	*9.5*	*8.5*	*R*	*R*	*Pct = 0.022*

R= resistant (showed little to no zone of inhibition) = 0; **Pc** = p value of clinical isolate; **Pct** = p value of clinical and typed isolate

Table 2: Mean diameter of inhibition zones at different extract concentrations against the typed isolate (ST ATCC 14028)

Extraction solvents	Dilution concentration					P value
	100 (mm)	***50 (mm)***	***25 (mm)***	***12.5 (mm)***	***0 (pure solvent)***	
7up	*R*	*R*	*R*	*R*	*R*	
Distilled water	*24*	*20*	*18.5*	*14*	*R*	*Pt = 0.020*
100% Ethanol	*10.5*	*R*	*R*	*R*	*R*	*Pct = 0.022*

R = resistant (showed little to no zone of inhibition) = 0; **ATCC** = American Type Culture Collection; **Pt** = p value of typed isolate; **Pct** = p value of clinical and typed isolate

Table 3: Inhibition zone diameters of the positive controls

Isolates	Amoxicillin-clavulanate(30mg)	Gentamicin (30mg)
Clinical isolate	*26mm*	25mm
Typed isolate (ST ATCC 14028)	22mm	26mm

Discussion

The present study tested aqueous infusion extracts as a way to validate the traditional remedy employed by a certain population. The 7up aqueous extract did not demonstrate any antimicrobial activity against both isolates of Salmonella typhi used in this study that validates the traditional use of 7up as an infusion medium for the leaves of Gossypium barbadense. There was virtually no zone of inhibition observed around the wells for the 7up extracts. This although might have resulted from some limitations encountered in the course of the experimental subjections.

Possible reasons behind the relatively poor results gotten from the experimental subjection of the extract might include: the solvent to sample ratio used in the preparation of the extracts may have been too low (25:1) as opposed the solvent to sample ratio usually used for infusion is 4:1 0r 16:1 (Abubakar and Haque, 2020). This may have resulted in the extracts being overly diluted as the infused extracts were not concentrated but used directly after extraction. The reverse was employed in a similar study carried out by Omoya (2020), the extraction process used was maceration with a solvent to sample ratio of 10:1. The extract was filtered after 72 hours and the filtrate dried in a rotary evaporator to concentrate the extract. The size of the wells may have been too small to contain enough of the dilute extract to produce antimicrobial activity.

7up is a lemon and lime flavored non-caffeinated soft drink that has been reformulated several times. One of its reformulations was done so it can be marketed as “100% natural” and this was achieved by eliminating the chelating agent calcium disodium EDTA and replacing sodium citrate with potassium citrate to reduce the beverage’s sodium content. This new formulation contains no fruit juice (Kuerig, 2016). A study conducted by Omoya (2020) demonstrated the antibacterial activity of lime-juice extract of the leaves of Gossypium hirsutum (a closely related specie from the same genus as Gossypium

barbadense) against Salmonella typhi. The extract not only demonstrated activity but also proved more effective than ciprofloxacin when used in vitro against clinical isolate and typed isolate (Salmonella typhi ATCC 14028) of Salmonella typhi. The study revealed that the Gossypium hirsutum extract not only showed high anti-salmonella showed to be concentration dependent in both evaluation methods used (agar well diffusion method and broth dilution method). The researcher also acknowledged that the lime juice used in the extraction process could have contributed to the high efficacy of the Gossypium hirsutum leaves extract. Gossypium hirsutum and Gossypium barbadense were reported to have certain similar active chemical compounds, although the compounds present in Gossypium barbadense are significantly less in concentration and components compared to Gossypium hirsutum (Ade-Ademilua & Okpoma, 2018). Juice from citruses like lime as well as lemon have been evaluated and reported to have several bioactive compounds such as ascorbic acid, saponins, alkaloids, phenols and flavonoids (Oikeh et al, 2016). Some of these classes of bioactive compounds like the phenols, alkaloids and flavonoids were also found present in Gossypium hirsutum (Egbuta et al., 2017). These compounds may have worked synergistically with that of the lime juice to produce high antimicrobial activity against Salmonella typhi in the study carried out by Omoya (2020). The lime (and lemon) flavored drink, 7up, did not demonstrate a similar effect when used as an extraction solvent in current study. This may have been a result of several factors; one possible reason of which may include: the 7up drink only contains lime flavor which may not be as potent as the fresh lime juice. Also, the other components that make up the drink like the sweetener, high fructose corn syrup (HFCS) may have contributed to the bacteria growth and hence, overpowering any effect possessed by the citric acid flavoring.

In this study, the innate anti-salmonella ability of Gossypium barbadense was demonstrated as the distilled water extracts showed activity against the Salmonella typhi isolates while the pure distilled water showed no activity against same isolates. However, some studies done with Gossypium barbadense as a focus or one of the subjects of study has reported that Gossypium barbadense has some antimicrobial activity (Tsoubou et al., 2015; Essein et al, 2011) but is significantly little especially when compared to its other prominent counterpart, G. hirsutum (Ade-Ademilua & Okpoma, 2018).

The amoxicillin-clavulanate positive control had an inhibition zone diameter measuring 26mm against the clinical isolate and 22mm against the typed isolate while the gentamicin positive control had an inhibition zone diameter of 25mm against the clinical isolate and 26mm against the typed isolate. The extract with the highest inhibition zone diameter – distilled water extract at dilution concentration of 100 measured 27mm against the clinical isolate and 24mm against the typed isolate, when compared against the positive control showed a slight difference in favour of the distilled water extract. The zones produced by the distilled water extract when compared to the positive controls were similar (±2mm). This shows that the highest dilution concentration of the distilled water extract has a considerably significant antimicrobial effect on Salmonella typhi, an effect similar to that of amoxicillin-clavulanate (30mg) and gentamicin (30mg). Compared to the activity of the other extracts, distilled water extracts showed far more anti-salmonella activity, demonstrating that distilled water may be a good extraction medium for the anti-salmonella properties of the leaves of Gossypium. barbadense. This is in agreement to the study carried out by Tsoubou et al., 2015.

Therefore, further studies should be carried out to specifically clarify the anti-salmonella effect of Gossypium barbadense and the interactions between its chemical compounds and the extraction solvents used or mentioned in the current study.

Conclusion

Only 58% (7) of the total 12 extracts showed activity against the Salmonella typhi clinical isolate while 42% (5) showed activity against the typed isolate. The highest zones of inhibition were obtained from distilled water extract with inhibition zone diameters of 27mm, 26mm, 24.2mm, 20mm for the clinical isolates and diameters of 24mm, 20mm, 18.5mm and 14mm for the standard strain; at dilution concentrations of 100mm, 50mm, 25mm and 12.5mm respectively. Some of the ethanoic extracts displayed signs of antimicrobial activity, more against the clinical isolate than the standard strain. The first three dilution concentrations of ethanol extract (100mm, 50mm, 25mm) showed activity against the clinical isolate while only the first concentration (100mm) showed significant activity against the typed isolate. The pure solution of ethanol showed insignificant activity against the two isolates. None of the 7up extract dilutions showed significant activity against any of the two isolates.

The importance of plants in medicine remains of greater relevance with the current shift to obtain drugs

from plants sources, resulting in the increase in attention given to the medical value of herbal remedies for safety, efficacy and economy. Distilled water itself (pure solution) showed no antimicrobial activity against both isolates. Although 7up showed no activity in this in vitro study, in vitro study using 7up extract in a more concentrated form is recommended as the extract used in this study may have been overly diluted. In vivo and molecular studies can be carried out to further assess if there is any basis behind the positive testimonies of the effectiveness of the use of 7up extract in the treatment of typhoid. Also, molecular studies can be carried out to test the interaction between the 7up and chemical constituents of Gossypium barbadense leaves.

In conclusion, Gossypium barbadense from this study shows innate anti-salmonella properties and distilled water seems to be a better vehicle for the extraction of the anti-salmonella properties of Gossypium barbadense leaves than 7up. It is therefore recommended that further studies be done to evaluate the scientific reason behind this observation; and also, on the interactions that occur between bioactive compounds present in Gossypium barbadense and pure water molecule/ions.

Conflict of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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