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The Use of Medicinal Plants as Alternatives for Typhoid Fever and Bacterial Gastroenteritis Therapy in Abwa-Mbagen, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author IWN designed the experiment, performed all the experiments and wrote the paper. Author BOA provided technical assistance. Author HOAO led and supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To assess the antibacterial activities and phytochemical potential of *Cochlospermum planchonii* (Apocynaceae), *Terminalia avicennoides* (Papilionaceae) and *Pericopsis laxiflora* (Papilionaceae) used traditionally against typhoid fever and bacterial gastroenteritis.
Study Design: In this study, hot water, hexane and methanol extracts obtained from the test plants were screened for phytochemicals according to standard procedures. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays were employed to determine the plant extracts susceptibilities to the test bacteria.
Place and Duration of the Study: Extraction was performed at the Chemistry laboratory, Benue State University while phytochemical screening and *in vitro* analyses were carried out at the Bacterial Research Division, National Veterinary and Research Institute Vom, Nigeria. All studies lasted for 12 months.
Methodology: Preparation of plant extracts, phytochemical analyses of the plant parts, agar well

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diffusion assay, determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was used to evaluate antibacterial activities. The Zones of Inhibition of various concentrations of extracts against test bacteria were also measured. Results were compared with standard antibiotic discs.

Results: Phytochemical studies reveal that while saponins were present in all the test plants anthraquinone was absent. Also, tannins, alkaloids, essential and volatile oils, flavonoids, phlobatannins, and steroids were identified in one or more of the plants. Findings from antibacterial activity reveal that Gram-positive bacteria were more sensitive to the extracts, by comparison to the Gram-negative bacteria.

The hexane extracts of both *C. planchonii* and *P. laxiflora* extracts inhibited *Bacillus cereus* at 0.165 µg ml^{-1}, while *T. avicennoides* hexane extract and decoction showed the least MIC of 0.33 µg ml^{-1} against *Stahylococcus aureus*.

Conclusion: The positive correlations of the results obtained here confirm the acclaimed ethnomedical uses, while also providing a potential source for discovering new pharmaceutical compounds with antibacterial activity.

Keywords: Cochlospermum planchonii; Terminalia avicennoides; Pericopsis laxiflora;typhoid fever; bacterial gastroenteritis; herbal medicine.

1. INTRODUCTION

In Africa as in several other countries globally, drug resistance presents an immense public health problem leading to increased morbidity and mortality [1,2,3,4]. It has been reported that about 80 – 90 % of the African population is dependent on herbal medicine for their primary health care [5]. Thus, many communities, especially in developing countries, depend on plants, which are known to produce an amazing array of novel biologically active phytochemicals [6], to cure many infectious diseases of man and livestock. Indeed, interest in plant-derived medicines is a re-emerging health care aid [7], and has assumed global proportions.

Resistance to known antibacterial synthetic agents results from the use and misuse of antibiotics, rendering once powerful antibiotics ineffective, and may, in turn, lead to treatment failure [8,9]. Consequently, with the decline in the development of novel antibiotics [10], a rise in the cost of prescription drugs [7], drug abuse, unavailability, toxic side effects and so on [11, 12,13], the use of combination therapy [14], or plants with antimicrobial properties [15,16] is thought to have immense potential in combating resistant pathogens. Equally, the potential for the development of new drugs depends on screening, evaluation, and documentation of herbal plants used in the treatment of diseases [17].

Bacterial infections are a significant cause of disease worldwide, and medicinal plants with antibacterial properties are often employed by traditional healers in therapy [18,19]. Shown in Fig. 1, is *Cochlospermum planchonii* (Hook) F. ex. Planch (Apocynaceae), a West African medicinal plant, the rhizomes and leaves are useful in the treatment of malaria, diabetes, trypanosomiasis [20,21,22,23,24,25]. In Mali, fresh roots of the plant have been reported to treat stomach disorder, typhoid fever but also urinary tract infection in combination with *Erythrina senegalensis* [26].

Terminalia avicennoides Guill, and Perr. (Combretaceae), Fig. 2; have been reported for the treatment of syphilis [27], wounds and ulcers. Aqueous root extracts of *T. avicennoides* possess antidiarrheal effects [28], thereby supporting its uses in the treatment of gastrointestinal disorders, reported among the Jukun, Idoma and Tiv tribes of Benue and Taraba states, Nigeria. The root extracts are also useful in multi-drug resistant *Salmonella typhi* treatment as reported [29]. Besides, the roots, which are used as chewing sticks, have been said to cure dental caries and also skin infections [30].

Among its numerous uses, *Pericopsis laxiflora* ex Baker van Meeuwen (Papilionaceae), indicated in Fig. 3, have been reported to cure cough and African trypanosomiasis [24]. Similarly, the plant is used in the therapy of malaria, stomachaches, ulcers, and gastritis in Cote D' Ivoire, Ghana and Nigeria [31,32,33,17]. Studies have shown activity against *Escherichia coli* Extended-Spectrum Beta-Lactamases (ESBL), *Klebsiella pneumoniae* [34] and also *Staphylococcus aureus* and *Shigella sp* [35].

Figure 1a: Whole plant of *C. planchonii in situ*

Figure 1b: Roots of *C. planchonii*

Figure 2a: Whole plant of *T. avicennoides in situ*

Figure 2b: Roots of *T. avicennoides*

Figure 3a: Whole plant of *Pericopsis laxiflora in situ*

Figure 3b: Roots of *Pericopsis laxiflora*

Bacterial gastroenteritis is prevalent in countries in both the developing and developed world, characterised by prolonged and (or) severe diarrhea that may result from water that is unsafe for drinking, poor sanitation and malnutrition [36]. According to World Health Organization, diarrheal disease is most common in children under five years with 1.7 billion

Table 1. Profile of the test plants used in this study

		Local names →						
Botanical Name	**Family**	**Tiv**	**Idoma**	**Igede**	**Etulo**	**Hausa**	**Traditional uses**	**References**
Cochlospermum Planchonii Hook. f ex Planch	Cochlospermaceae	Kpavande		Opiampir	Akikpopo/ Ishawe	Rawaya, Belge	Malaria, diabetes, trypanosomiasis, diarrhoea, typhoid fever, sexually transmitted infections	[20,21,22,23,24,27]. [28,29,30]
Terminalia avicennoides Guill & Perr.	Combretaceae	Kuegh	Okwo		Mkulo	Baushi	Wounds, ulcers gastrointestinal disorders, syphilis	
Pericopsis laxiflora Benth ex Baker Van Meeuwen	Papilionaceae	Jiagba	Agama	Odagbila	Jaagba	Bakin, Makarfo, Bajini	Cough, African trypanosomiasis	[24,31,32,33,16,34, 35].

The details for the local names of the plants were obtained from [39]

recorded cases yearly and 520 000 mortalities, thus ranking the second biggest causes of death [37]. Typhoid fever is caused by *Salmonella typhimurium*, and causes severe gastroenteritis [38]. Traditional Medical Practitioners (TMP) at Abwa-Mbagen, Benue State, Nigeria claim that whole roots of the test plants (Table 1) are used in combination therapy for the cure of typhoid fever, bacterial gastroenteritis and other microbial diseases. This paper therefore evaluates the medicinal plants (Table 1) individually and in combination for their antibacterial properties, within the context of their reported claims by the TMP's from Abwa-Mbagen, Nigeria, as this has not been previously investigated.

2. MATERIALS AND METHODS

2.1 Ethnobotanical Survey

Medicinal uses of the test plants were collected from randomly selected herbalists in Abwa-Mbagen, Benue State, Nigeria. With the assistance of the TMP's, the plants were collected, identified by a taxonomist and voucher specimens deposited at the Herbarium of the Plant Section, Department of Biological Sciences, Benue State University Makurdi, Nigeria.

2.2 Test Bacteria Species

Clinical isolates of *Salmonella typhi* and *Pseudomonas aeuroginosa*, employed in the study were obtained from the Federal College of Medical Laboratory Technology, Vom, however, typed cultures of *Escherichia coli* ATCC (25922), *Staphylococcous aureus* ATCC (29213) and *Bacillus cereus* ATCC (11778) were supplied by National Veterinary and Research Institute (NVRI), Vom, Nigeria.

2.3 Pre-extraction Preparation of Medicinal Test Plants

Whole root samples of *C. planchonii*, *T. avicennoides* and *P. laxiflora* were dried at room temperature (29 + 2^{o}C), pulverised using a pestle and mortar, separately sieved and labelled in airtight, sterile polythene bags until required for use.

2.4 Soxhlet Extraction

Using the Soxhlet method, a 50 g portion each of pulverised root samples from the respective plant materials were filled separately in the thimble and extracted, successively with 500 ml of 99% hexane and 95% methanol for six hours as described [40]. The organic extracts were concentrated by evaporating to dryness at room temperature in a dry air current, after which they were stored in labelled specimen bottles in desiccators.

2.4.1 Decoction

A 50 g portion each of the pulverised samples from the test plants was measured into 200 ml sterile distilled water in a conical flask and heated to 80 – 100^{o}C for 15 – 20 minutes in a similar method as with the TMP. The resulting expressed were filtered using three layers of muslin cloth and later passed through Whatman filter paper No. 1. The extracts were concentrated in a sand bath maintained at 60^{o}C.

2.4.2 Qualitative Phytochemical screening

Extraction of active ingredients from the test plants was carried out using standard procedures [41]. Small portions of each crude extract were subjected to appropriate phytochemical analysis and the following components were screened for: saponins, tannins, alkaloids, volatile and essential oils, flavonoids, phlobatannins, steroids and reducing sugar. The results were read based on either the formation of colour or precipitate, on the inclusion of suitable reagents.

2.5 Antibacterial Activity Evaluation

We next investigated the antibacterial effect of the plant extracts on test bacteria using the agar well diffusion method [42] on Mueller-Hinton agar (MHA, Merck) and paper disk diffusion test for the control. Briefly, 0.5 ml of a 24-h broth culture (10^{6} cfu ml^{-1}) of the bacteria cultured in broth was aseptically introduced and evenly streaked on the surface of the sterile Mueller Hinton Agar (MHA) plates. Eight wells of 5.00 mm diameter were aseptically bored on agar plates using a sterile cork borer. Fixed volumes (0.5 ml) of the extract were introduced into the wells in the plates using a Pasteur's pipette. This was allowed for 2 h at room temperature for absorption and diffusion to take place. The plates were incubated at 37 ± 2°C for 24 h. These studies were performed in triplicates and the mean values considered. Dimethyl sulphooxide (DMSO) was used on one well as negative control while Ofloxacin disc was used for the purpose of comparison. Antibacterial activities were calculated by measuring the zones of

inhibition of bacterial growth with a transparent ruler in mm and comparing with control.

2.6 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

We used the broth dilution assay [43] with slight modification to determine the minimum inhibitory concentrations (MIC) of the test bacteria against test plant samples. A loopful of the bacterial culture was inoculated in broth and incubated at 37 ± 2^{o} C for 24 hours. A fresh broth (20 ml) was then seeded with 0.25 ml of the 24 h broth cultures, adjusted to a 0.5 MacFarland standard, and a two-fold serial dilution method was followed. After incubation, the tube with no visible growth of the bacteria was taken as the MIC of the test sample expressed in µg ml^{-1}. For the minimum bactericidal concentration (MBC), a loopful of broth was collected from those tubes that did not show any growth and inoculated on sterile Mueller Hinton Agar by streaking. The inoculated plates were then incubated at 37 ± 2°C for 24 h. The concentration at which no visible growth was observed was noted as the MBC. Two controls were maintained for each test batch.

3. RESULTS

3.1 Phytochemical Screening

The results for phytochemical studies (Table 2) reveal the presence of saponins in all the three medicinal plants. Tannins, flavonoids, steroids and reducing sugar were detected only in *C. planchonii* and *T. avicennoides*. These compounds are indicative of the potential therapeutic value of the plants in which they appear [44,45,46].

3.2 Antibacterial Activity of Test Plants is Concentration Dependent

Antibacterial activity of *C. planchonii* on test bacteria is summarised in Supplementary Fig. 1a-1c). No zone of inhibition was observed for the decoction (Supplementary Fig. 1c) and hexane extracts of *C. planchonii* (Supplementary Fig. 1a) against *E. coli* and *S. typhi*, thus agreeing with MIC test results. Though time-kill curve assays on the extracts were not performed, it was noted that the higher the concentration of the extract, the greater the zone of inhibition for test bacteria that showed sensitivities. Thus the hexane extract of *B. cereus* had the largest inhibition zone of 21 mm at 333.33 µg ml^{-1} and decreased to 8.5 mm with a reduced concentration of 2.60 µg ml^{-1} (Supplementary Fig. 1a). Surprisingly, the methanol extract of *C. planchonii* did not show any inhibition of *P. aeuroginosa* at all the concentrations tested (Supplementary Fig. 1b).

Similar to *C. planchonii*, both the hexane extracts and a decoction of *T. avicennoides* revealed no zones of inhibition when tested against *E. coli* and *S. typhi*, but in addition to *P. aeuroginosa* (Supplementary Fig. 2a-c). It was however interesting to note that the hot water extract (decoction) of *T. avicennoides* recorded the most extensive zones of inhibition of 25 mm against *S. aureus* (Supplementary Fig. 1c), but as the concentration of the extract decreased to 2.60 µg ml^{-1}, so did the zone of inhibition decrease to 7.0 mm. Equally, the hexane extract of *T. avicennoides* showed the highest inhibition zone of 12.25 mm recorded by *B. cereus* at 333.33 which was the widest (Supplementary Fig. 2a), recorded for test bacteria. Correspondingly, the methanol extract of the roots of *T. avicennoides* showed antibacterial activities to all the test bacteria with the widest zone of inhibition of 22.00 mm at 333.33 µg ml^{-1} on *S. aureus*, and *S. typhi* showed the least zone of inhibition of 3.00 mm at a concentration of 5.21 µg ml^{-1}.

The decoction of *P. laxiflora*, did not inhibit growth for all bacteria tested (Supplementary Fig. 3c). Similarly, the methanol extract of *P. laxiflora* that showed the least activity did not result to growth inhibition in *E. coli* and *S. typhi* (Supplementary Fig. 3b) Only the high concentrations of 333.33 - 83.33 µg ml^{-1} demonstrated some growth inhibition to *B. cereus*, *S. aureus* and *P. aeuroginosa*. On the contrary, for unknown reasons, only *S. typhi* was not inhibited by the hexane extract of *P. laxiflora* while *S. aureus* was inhibited by 24.00 mm at 333.33 µg ml^{-1} (Supplementary Fig. 3a).

The concoction of the extracts from the test plants inhibited only the Gram-positive bacteria *B. cereus* and *S. aureus* at intermediate (20.34 µg ml^{-1}) to high concentrations 333.33 µg ml^{-1} with the widest zone of inhibition of 21.00 mm observed by *B. cereus* (Supplementary Fig. 4).

3.3 MIC and MBC

Data for antibiotic susceptibility is shown for *C. planchonii* (Table 3), *T. avicenoides* (Table 4) and *P. laxiflora* (Table 5) extracts individually

against the test bacteria. Results reveal that by comparison to all the other test bacteria, *S. typhi* was less susceptible, inhibited by MIC and MBC values of 10.42 and 41.67 µg ml^{-1} respectively (Table 3), while *B. cereus* was the most vulnerable organism with MIC and MBC values of 0.165 and 0.33 µg ml^{-1} respectively (Table 3 and 5).

Surprisingly, decoction of *P. laxiflora* did not inhibit any of the test organisms (Supplementary Fig. 3c), yet the most susceptible organism was the hexane extract that inhibited *S. aureus* at a concentration 0.33 µg ml^{-1} (Table 5). The least susceptible organism was *B. cereus* and *P. aeuroginosa* with MIC and MBC of 10.42 and 41.67 µg ml^{-1} respectively as we observed with the methanol extract.

As indicated in Table 6, the concoction of the test plants inhibited only the Gram-positive bacteria.

Table 2. Qualitative phytochemical analysis of the test plants

Phytochemical Test/ Specimen	*C. planchonii Hook. f ex Planch*	*T. avicennoides Guill & Perr.*	*P. laxiflora Benth Van*
Saponins	+	+	+
Tannins	+	+	-
Alkaloids	-	+	-
Volatile Oils	-	+	-
Essential oils	+	-	-
Flavonoids	+	+	-
Phlobatannin	-	+	-
Steroid	+	+	-
Reducing sugar	+	+	-
Anthraquinone	-	-	-

Key: + = presence, - = absence

Table 3. MIC/MBC (µg ml^{-1}) of *C. planchonii* extracts

Extract/MIC/MBC value	*E. coli*	*B. cereus*	*S. aureus*	*P. aeuroginosa*	*S. typhi*
HE					
MIC	-	0.165	0.65	1.30	-
MBC	-	0.33	1.30	2.60	-
ME					
MIC	2.60	0.33	0.65	-	10.42
MBC	10.42	0.65	2.60	-	41.67
DEC.					
MIC	-	0.65	0.65	-	-
MBC	-	2.60	2.60	-	-

Activity key: - shows no activity, HE: Hexane extract, ME: Methanol extract, DEC.: Decoction

Table 4. MIC/MBC (µg ml^{-1}) of *T. avicennoides* extract against test organisms

Extract/MIC/MBC value	*E. coli*	*B. cereus*	*S. aureus*	*P. aeuroginosa*	*S. typhi*
HE					
MIC	-	1.30	1.30	-	-
MBC	-	10.42	5.21	-	-
ME					
MIC	10.42	2.60	0.33	1.30	0.65
MBC	20.34	10.42	1.30	5.21	2.60
DEC.					
MIC	-	0.165	0.33	-	-
MBC	-	0.33	0.65	-	-

Activity key: - shows no activity, values are in µg/ml

Table 5. MIC/MBC (µg ml^{-1}) of *P. laxiflora* extract on test organisms

Extract/MIC/MBC value	*E. coli*	*B. cereus*	*S. aureus*	*P. aeuroginosa*	*S. typhi*
HE					
MIC	1.30	0.165	0.33	2.60	-
MBC	5.21	0.33	0.65	10.42	-
ME					
MIC	-	10.42	2.60	10.42	-
MBC	-	41.67	10.42	41.67	-
DEC.					
MIC	-	-	-	-	-
MBC	-	-	-	-	-

Activity key: - no inhibition

Table 6. MIC/MBC (µg ml^{-1}) of the concoction of the test plants against test bacteria

Extract/MIC/MBC value	*E. coli*	*B. cereus*	*S. aureus*	*P. aeuroginosa*	*S. typhi*
MIC	-	0.65	0.65	-	-
MBC	-	2.60	2.60	-	-

Activity key: - shows no activity

4. DISCUSSION

In this study, the antimicrobial and phytochemical properties of the extracts of *T. avicennoides*, *C. planchonii* and *P. laxiflora* extracts using hexane, methanol and hot water were evaluated against both clinical strains and reference bacteria, *in vitro*.

Plant antimicrobials are not used as systemic antibiotics at present because of their low level of activity against Gram-negative bacteria as we have shown. The MBC values obtained for all the extracts against the pathogens are higher than MIC, indicating that the extracts are bacteriostatic at low concentrations and bactericidal at higher concentrations. Since most of the traditional preparations lack specific levels, this may account for the use of large quantities of the extracts by TMP for treatment of their patients.

Results from the study identified several phytochemical components (Table 2), some results of which correlate with studies of [47] in which essential oil was present in the rhizome of *C. planchonii*, and also [48] that identified the presence of saponins, tannins, alkaloids and volatile oil in the roots of *T. avicennoides*.

The plant extracts used in this study showed a stronger inhibitory effect on the Gram-positive bacteria namely *S. aureus*, *B. cereus* by comparison to Gram-negative *E. coli*, *S. typhi* and *P. aeuroginosa* (Supplementary Figs. 1-3). The extracts of *P. laxiflora* were interestingly inhibitory against both Gram-negative and Gram-positive bacteria, an indication of broad-spectrum compounds in the plants, though *P. aueroginosa* and *S. typhi* did not show any sensitivity to these extracts. *B. cereus* and *S. aureus* were the most susceptible bacteria to all the plant extracts while *E. coli* and *S. typhi* were the most resistant to all the extracts confirming the work of [49] that Gram-negative bacteria were more resistant to plant extract when compared with Gram-positive bacteria. In agreement with this, [18] observed that no extract from fifty plants was active against Gram-negative strains. These Gram-negative bacteria have an effective permeability barrier, comprised of the outer membrane, which restricts the penetration of amphipathic compounds and multidrug-resistant pumps that extrude toxins across this barrier. It is therefore noteworthy that the hexane extract of *P. laxiflora* did not show antibacterial activity against both Gram-positive and Gram-negative bacteria.

The presence of the phytochemical constituents could explain the acceptable standard inhibition zone for sensitive antibacterial activities shown by these plants. Previous reports have demonstrated the antidiarrheal activity of tannins [50] and flavonoids [51] and Saponins [52]. These compounds were screened for in this study and saponins was detected in all the plant materials. This may illustrate the importance of saponins in antidiarrheal property of medicinal

plants in this study. Both *C. planchonii* and *T. avicennoides* tested positive for tannins, thus ascertaining their medicinal uses. The antibacterial activity of *C. planchonii* could be attributed to the presence of flavonoids, which is ascribed to their ability to compare with extracellular and soluble protein as well as their ability to complex with bacterial cell wall and also suggested that more lipophilic flavonoids exert antimicrobial activity by disrupting microbial cell membrane [53]. Though TMP's at Abwa-Mbagen have applied the concoction of the plants in the therapy of typhoid fever, which they have found useful, data obtained from our study revealed that a clinical strain of *S. typhi* was not inhibited using the decoction of the plant extracts when used singly or in combination *in vitro* (Supplementary Fig. 4). But the methanol extract of *T. avicennoides* showed some activity against *S. typhi* (Supplementary Fig. 2b), notwithstanding, the identification of various phytochemicals and broad spectrum of activity, when compared against the three extracts, validates the utilization of these plants in treating bacterial infections, hence the continued patronage of the local community to the TMP's which could result from other bacteria besides *S. typhi*.

5. CONCLUSION

Though further studies are required, results obtained from the research have contributed to a better understanding of the medicinal plants employed in treating pathogens implicated in typhoid fever and bacterial gastroenteritis and may serve as sources of a novel antibacterial agent in the future.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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