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Screening of Eight Plants Used In Folkloric Medicine for the Treatment of Typhoid Fever

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ABSTRACT

Aqueous, ethanol and chloroform extracts of some plants used Igala folkloric medicine of North-Central Nigeria for the treatment of typhoid fever were investigated for their antibacterial properties against Salmonella typhi. Screening for the antibacterial activity was carried out by using a modification of the agar well diffusion technique. The extracts that showed significant antibacterial activity were further investigated for their Minimum Inhibitory Concentrations [MIC]. The aqueous extract of D. oliveri and the ethanol extract of M. indica had MIC of ≤12.5mg/ml which was comparable to that of gentamycin and better than those of amoxicillin and chloramphenicol, 25mg/ml and 100mg/ml respectively. However, the aqueous extract of N. latifolia which had an MIC of 25mg/ml had the widest zone of inhibition of all the extracts investigated from 25mg/ml to 100mg/ml concentration of the extracts. Thus weight for weight, the aqueous extract of N. latifolia was the most potent at higher concentrations. The results obtained validate the use of A. occidentale, A. vogelii Planch, D. oliveri, M. indica and N. latifolia in Igala folkloric medicine for the treatment of typhoid fever.

Keywords: Igala, Nigeria, *S. typhi*, typhoid fever, folkloric medicine

INTRODUCTION

Typhoid fever is caused by *Salmonella typhi* [Gianella, 2006]. Typhoid fever remains an important public health problem particularly in developing countries with approximately 10 million cases and it results in about 700,000 deaths annually [Kariuki and Hart, 1997; Wasfy *et al.*, 2000; Threlfall and ward, 2001]. In Nigeria, typhoid fever is among the major widespread diseases affecting both young children and adult in their productive years. This is as a result of

many interrelated factors such as increased urbanization, inadequate supplies of potable water, overburdened healthcare delivery systems and indiscriminate use of antibiotics that contribute to the development of antibiotic resistant *Salmonella typhi [*Adeleye and adetosoye, 1993; Talabi, 1994].

The antibiotics that form the mainstay therapy for typhoid fever patients in developing countries are chloramphenicol, ampicillin, amoxicillin and cotrimoxazole. Resistant strains of *S. typhi* to these antibiotics have emerged and continues to be of clinical significance. The frequent failure with empirical therapy observed in some hospitalized typhoid fever patients is as a result of widespread circulation antibiotic resistant strain of *S. typhi* [Saha *et al.*, 1997]. Studies in Nigeria have shown that from 1997 to 2003, 80% of isolates of *Salmonella typhi* were multiple drug resistant [MDR], chloramphenicol being the most resisted antibiotic [83%] followed by ampicillin [81.7%]. Also the prevalence of chloramphenicol resistant isolates increased gradually from 72.4% in 1997 to 89.2% in 2003. Similar trends were also recorded for other antibiotics [Akinyemi *et al.,* 2005]. Other studies confirm the increase in circulation of MDR- *Salmonella typhi* isolates over a relatively short period [Rowe *et al.,* 1991; Oboegbulam *et al.,* 1995; Agbonlahan *et al.,* 1997].

Even before the discovery of modern antibiotics and other chemotherapeutic agents, traditional medicine has served as man's resort when attacked by infective agents such as bacteria and fungi [Crafton, 1983]. Herbal medicine has still maintained its popularity in all regions of developing world and its use is rapidly spreading in industrialized countries [Patrick, 2002]. Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components [Shariff, 2001]. Recent work revealed the potential of several herbs as sources of drugs [Parekh and Chanda, 2007]. The first line of treatment for 60% of children with high fever in Ghana, Mali, Nigeria and Zambia resulting from typhoid fever and malaria is the use of herbal medicine [WHO, 2005].

The Igalas, in Kogi State, North-Central Nigeria use medicinal plants for the treatment of typhoid fever, but little work has been done to systematically identify and document these plants, study their pharmacological properties, domesticate and cultivate them, despite the fact that several of these plants face the danger of being lost due to the menace of deforestation and reluctance of people to venture into forest to harvest them. Another real danger is losing this indigenous knowledge when the custodians die. Also there is still an urgent need to identify novel substances that are active towards pathogens with high resistance [Recio *et al*., 1989, Cragg *et al*., 1997, Barbour *et al*., 2004].

Some of the plants used in Igala folkloric medicine for the treatment of typhoid fever include *Anacardium occidentale, Anthocleista vogelii* Planch, *Alchornea cordifolia, Cassia sieberiana, Daniela oliveri, Mamgifera indica, Nauclea latifolia* and *Triplochyton scleroxylon*. Their mode of preparation and administration in Igala folkloric medicine are as presented in Table 1.

Table 1: Some Plants used in Igala Folkloric Medicine for the Treatment of Typhoid Fever

Botanical name	Local name	Method of preparation and admistration	Other uses
Anarcadium occidentale	Opigolo	The leaves or bark boiled in water, the bark can also be soaked in alcohol, and is drunk over a period of time depending on the severity	The bark is used for the treatment of ulcer and dysentery and the leaves as antifungal
Anthocleista vogelii Planch	Odogwu	The leaves boiled in water and is drunk over a period of time depending on the severity.	Applied as poultice on swellings and to cleanse wound. Also used as an antifungal.
Alchornea cordifolia	Oyi	The leaves boiled in water and is drunk over a period of time depending on the severity.	Treatment of stomach ache and other stomach disorders and sexually transmitted diseases. Used by women that have just given birth
Cassia sieberiana	Itolo	The leaves or bark boiled in water, and is drunk over a period of time depending on the severity. The crushed leaves are soaked in alcohol and taking for a shorter period	Used for the treatment of malaria. Roots used for the treatment of convulsion
Daniela oliveri	Oda	The leaves boiled in water and is drunk over a period of time depending on the severity.	Malaria and yellow fever treatment
Mamgifera indica	Umagolo	The leaves or bark boiled in water, and is drunk over a period of time depending on the severity. The leaves or macerated bark can also be taken as tinctures in alcohol	Treatment of malaria, diabetes and other diseases.
Nauclea latifolia	Ogbayi	The leaves boiled in water and is drunk over a period of time depending on the severity.	Treatment of malaria, diarrhea, dysentery, and pneumonia. Applied as poultice cleanse wound
Triplochyton scleroxylon	Uwewe	The leaves boiled in water and is drunk over a period of time depending on the severity.	Treatment hypertension and ulcer. Applied as poultice cleanse wound

EXPERIMENTAL SECTION

The plants were collected from various locations in the Igala speaking areas of Kogi State, North-Central Nigeria. They were identified by Prof. COC Agwu of the Biological sciences Department, Kogi State University, Anyigba, Nigeria. The test organism: Clinical isolate of *Salmonella typhi* and was obtained from the Microbiology Department of Nigerian Institute of Medical Research [NIMR], Yaba, Lagos, Nigeria. Reagents and media were obtained from reputable names like BDH Poole, England and Lab M ltd. Lancashire, England.

Preparation of Plant Extracts

The plant materials were washed and then air dried at room temperature for some days. They were pulverized using high speed Creston grinder.

For the aqueous extract, 100g of the pulverized plant sample was macerated in 750 ml aliquot of distilled water for 24 hours. This was then filtered through Whatman No 1 filter paper using a Speedvac vacuum pump. The filtrate was then evaporated to dryness in a water bath to obtain the aqueous crude extract [yield = % starting material].

To obtain the chloroform and ethanol extracts, 100g of the pulverized plant sample was macerated in 6 volume [w:v] chloroform-ethanol [2:1], shaken vigorously and allowed to stand for 24 hours. This was then filtered through Whatman No 1 filter paper. The filtrate was shaken vigorously with 0.2 volume of distilled water to obtain two distinct layers. The upper and lower layers were then separated into different beakers and evaporated to dryness in a water bath to obtain the ethanol extract [yield = % starting material] and the chloroform extract [yield = % starting material] respectively.

Antibacterial Screening

The antibacterial activity of the aqueous, ethanol and chloroform extracts of the plant sample was determined using a modification of the agar well diffusion technique described by Odama *et al.* [1986] and Perez *et al.* [1990]. The extract that showed antibacterial activity of $\geq$ 10mm clear zone of inhibition at 25mg/ml concentration were further investigated to determine their Minimum Inhibitory Concentration [MIC].

The MIC was determined using the serial dilution method. Four test tubes were prepared for each of the extracts. The test tubes contained 3ml of 12,5mg/ml, 25mg/ml, 50mg/ml and 100mg/ml of reconstituted crude extracts in nutrient broth agar. A drop [0.025ml] of the standardized bacteria was then suspended in each of the test tubes. Similarly, four test tubes were prepared for each of the standard antibiotics, gentamycin, amoxicillin and chloramphenicol, used as control, and inoculated with the standardized bacteria. They were then incubated at 37^{o}C for about 15 to 24 hours. The experiment was carried out in triplicates. A test tube containing 3ml of nutrient broth agar inoculated with the bacteria was used to monitor the growth of the microorganism by reading the absorbance at 540nm for 18 hours at every 3 hour interval. The MIC was determined as the lowest concentration of crude extracts that inhibited visible growth [turbidity] of the test organism after 15 hours.

RESULTS

Only two of the extracts had appreciable activity on the bacteria at low concentration of 12.5mg/ml. At higher concentrations the activity of the extracts against the bacteria increased in a concentration dependent manner. However, the aqueous extracts of *M. indica* and *C. siberinea*,

the ethanol extract of *T. scleroxylon* and the chloroform extracts of *A. cordifolia*, *D. oliveri* and *T. scleroxylon* were inactive at all concentrations investigated. Figure 1 shows that the active growth of the bacteria stopped at 15 hours, therefore the MIC of the plant extracts was determined as lowest concentration that inhibited visible growth [turbidity] of the bacteria after 15 hours of incubation.

Table 2: Antibacterial activity of aqueous extract of plant samples on *S. typhi*

	Zone of Inhibition [mm]			
PLANT/CONTROL	**12.5mg/ml**	**25mg/ml**	**50mg/ml**	**100mg/ml**
Anarcadium occidentale	8*	10	12	14
Alchornea cordifolia	8*	8*	12	14
Anthocleista vogelii Planch	8*	12	16	18
Cassia sieberiana	8*	8*	8*	8*
Daniela oliveri	10	13	15	18
Mamgifera indica	8*	8*	8*	8*
Nauclea latifolia	8*	15	17	19
Triplochyton scleroxylon	8*	9	12	14
Amoxicillin	12	12	15	17
Chloramphenicol	8*	8*	8*	15
Gentamycin	18	18	20	25

* The diameter of the cork borer used was 8mm; it thus means there was no clear zone of inhibition

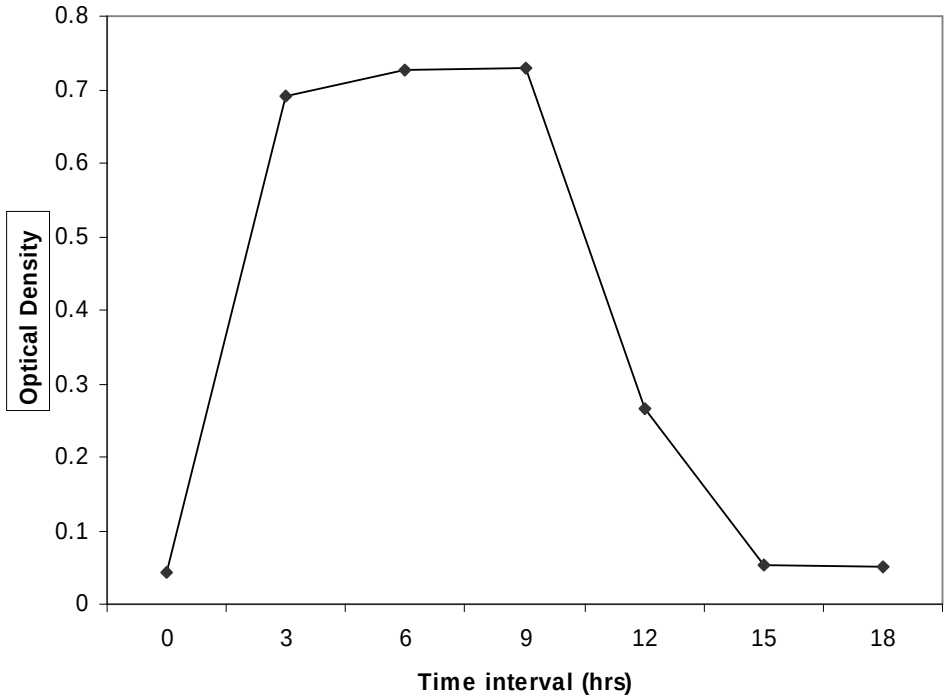

Fig 1: Growth rate of Salmonella typhi using the measurement of their optical density at 540nm

The antibacterial activity of the aqueous extracts was exerted for most of the extracts beginning from 25mg/ml concentration. The antibacterial activity aqueous extracts of *D. oliveri* and *N. latifolia* were consistently higher that those of chloramphenicol and amoxicillin but slightly lower than that of gentamycin. Even though *N. latifolia* had the widest zone of clear inhibition at 25 -100 mg/ml concentrations, D. *oliveri* had the lowest MIC of ≤12.5mg/ml which was comparable to those of amoxicillin and gentamycin.

Table 3: MIC of aqueous extract of plant samples on *S. typhi*

PLANT/CONTROL	12.5mg/ml	25mg/ml	50mg/ml	100mg/ml
Anarcadium occidentale	+	-	-	-
Anthocleista vogelii Planch	+	-	-	-
Daniela oliveri	-	-	-	-
Nauclea latifolia	+	-	-	-
Triplochyton scleroxylon	+	-	-	-
Amoxicillin	-	-	-	-
Chloramphenicol	+	+	+	-
Gentamycin	-	-	-	-

+: growth
-: no growth

Table 4: Antibacterial activity of ethanol extract of plant samples on *S. typhi*

	Zone of Inhibition [mm]			
PLANT/CONTROL	12.5mg/ml	25mg/ml	50mg/ml	100mg/ml
Anarcadium occidentale	8*	8*	15	17
Alchornea cordifolia	8*	8*	10	12
Anthocleista vogelii Planch	8*	12	14	16
Cassia sieberiana	8*	8*	11	12
Mamgifera indica	11	12	14	18
Nauclea latifolia	8*	10	12	12
Triplochyton scleroxylon	8*	8*	8*	8*
Amoxicillin	12	12	15	17
Chloramphenicol	8*	8*	8*	15
Gentamycin	18	18	20	25

* The diameter of the cork borer used was 8mm; it thus means there was no clear zone of inhibition

Table 5: MIC of ethanol extract of plant samples on *S. typhi*

PLANT/CONTROL	12.5mg/ml	25mg/ml	50mg/ml	100mg/ml
Anarcadium occidentale	+	-	-	-
Anthocleista vogelii Planch	+	-	-	-
Mamgifera indica	-	-	-	-
Nauclea latifolia	+	-	-	-
Amoxicillin	-	-	-	-
Chloramphenicol	+	+	+	-
Gentamycin	-	-	-	-

+: growth; -: no growth

Most of the ethanol extracts started exhibiting their antibacterial activity from 50mg/ml concentration. *M. indica* which had the widest diameter zone of inhibition of all the ethanol extracts at 100mg/ml also had the lowest MIC of ≤12.5mg/ml which was comparable to those of amoxicillin and gentamycin.

The chloroform extracts were virtually ineffective against the bacteria at 12.5mg/ml and 25mg/ml concentration and so there was no need for further test to determine the MIC. The chloroform extracts of *A. occidentale, C. sieberiana* and *N. latifolia* showed some activity against the bacteria at 100mg/ml but were lower than those of the standard antibiotics used.

Table 6: Antibacterial activity of chloroform extract of plant samples on *S. typhi*

	Zone of Inhibition [mm]			
PLANT/CONTROL	**12.5mg/ml**	**25mg/ml**	**50mg/ml**	**100mg/ml**
Anarcadium occidentale	8*	9	12	14
Alchornea cordifolia	8*	8*	8*	8*
Anthocleista vogelii Planch	8*	9	10	12
Cassia sieberiana	8*	8*	11	14
Daniela oliveri	8*	8*	8*	8*
Mamgifera indica	9	9	9	12
Nauclea latifolia	8*	9	11	14
Triplochyton scleroxylon	8*	8*	8*	8*
Amoxicillin	12	12	15	17
Chloramphenicol	8*	8*	8*	15
Gentamycin	18	18	20	25

* The diameter of the cork borer used was 8mm; it thus means there was no clear zone of inhibition

DISCUSSION AND CONCLUSION

All the plants exhibited antibacterial activities against *S. typhi* with a clear zone of inhibition especially at 100mg/ml concentration. It also appears as is the isolate of the *S. typhi* used is a chloramphenicol resistant strain as it was completely insensitive to chloramphenicol at 12.5 mg/ml to 50 mg/ml concentration, whereas it was susceptible to the gentamycin and to a lesser extent amoxicillin.

At 12.5mg/ml, only the aqueous extract of *D. oliveria* and ethanol extract of *M. indica* showed appreciable activity against the test organism. This suggests that these extracts are the most potent of all plant extract studied. However, weight for weight, the aqueous extract of *N. latifolia* was more potent as from 25mg/ml concentration and above. Interestingly, these three extracts have activities that compares favourably to the activity of amoxicillin, a standard antibiotic against typhoid fever, this underscores the importance of the plant extract as potent anti-typhoid agents.

The ethanol extract of *A. occidentale* did not show any clear zone of inhibition at 25mg/ml in the agar well diffusion technique, but the serial dilution technique revealed that it had an MIC of 25mg/ml. this suggests that the serial dilution technique is a better method of determining the activity of crude extracts against microorganisms. The aqueous extract of *A. occidentale* had a clear zone of inhibition of 10mm at 25mg/ml and an MIC of 25mg/ml. this is comparable to the

activity of amoxicillin, but slightly lower than that of gentamycin. This validates the application of *A. occidentale* in Igala folkloric medicine for the treatment of typhoid fever. The chloroform extract was not active, implying that polar extraction is best for this plant in the treatment of typhoid fever.

The extracts of *A. cordifolia* were not active against the test organism at concentration up to 25mg/ml, but from 50 mg/ml, the water and ethanol extract started to show some activities against the test organism. The activity was however lower than those of the standard antibiotics. This in itself does not indicate that *A. cordifolia* is not effective in the treatment of typhoid fever, but that it may require higher concentration for it to be effective.

The polar extracts of *A. vogelii* Planch were very effective against the test organism starting from 25mg/ml concentration with value similar to that of a standard antibiotic. The polar extracts also had MIC of 25mg/ml. these suggest that there is a pharmacological rationale for the use of *A. vogelii* Planch in Igala folkloric medicine for the treatment of typhoid fever.

The aqueous extract of *C. sieberiana* was completely ineffective against the test organism. The chloroform and ethanol extracts however exerted some antibacterial activity from 50mg/ml concentration. This indicates that at higher concentrations, organic extracts of *C. sieberiana* may be effective against *S. typhi*. This may also explain why the plant is administered as tincture in alcohol in Igala folkloric medicine.

From Tables 2 and 3, we can see that the aqueous extract of *D. oliveri* was very potent and in fact the most potent of the extracts studied at the lowest concentration investigated. However, the organic extracts were completely ineffective against the test organism. The antibacterial activity of the aqueous extract was comparable to that of gentamycin but greater than those of chloramphenicol and amoxicillin. This show that the use of this plant sample in the treatment of typhoid fever in Igala folkloric medicine may be borne out of some traditional experiments in time past and this study confirm the validity of the claims.

The ethanol extract of *M. indica* had 12mm clear zone of inhibition at 25mg/ml concentration, this is comparable to the activity of the standard antibiotics used. It also had an MIC of ≤12.5mg/ml, which is the same as gentamycin but lower than that of amoxicillin. This shows that the extract is very potent as an anti-typhoid agent, and validates its use in Igala folkloric medicine as an anti-typhoid agent. It also agrees with the findings of Srinivasan *et al.* [2001] who said a component of *M. indica* displays a high antibacterial activity against gram negative bacteria.

The aqueous extract of *N. latifolia* had the widest clear zone of inhibition of all extracts [w:w] at all concentrations except at 12.5mg/ml where it was inactive against the test organism. The MIC of the extract, 25mg/ml, further proves that it is ineffective against the test organism at concentrations below 25mg/ml. it is the only extract that had wider clear zones of inhibition than amoxicillin at 25mg/ml to 100 mg/ml concentrations. This underscores its potency as an anti-typhoid agent. This provides the pharmacological basis for the use of this plant sample in Igala folkloric medicine for the treatment of typhoid fever.

The ethanol and chloroform extracts of *T. scleroxylon* were completely ineffective against the test organism at all concentrations. The aqueous extract also showed some form of activity at concentrations of 50mg/ml and above. This suggests that the plant is not very effective against *S. typhi* at low concentrations.

From this investigation, we can conclude that there is a pharmacological rationale for the use of materials from plants like *A. occidentale, A. vogelii* Planch, *D. oliveri, M. indica* and *N. latifolia* in Igala folkloric medicine for the treatment of typhoid fever. Further studies is however required in order to validate [or otherwise] the potential of *A. cordifolia, C. siberinea* and *T.* scleroxylon as anti-typhoid agents.

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