Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2011, 2 (1): 222-227

Der Pharmacia Sinica

ISSN: 0976-8688
CODEN (USA): PSHIBD

A comparative study of the antibacterial activity of aqueous ethanol and chloroform extracts of some selected medicinal plants used in Igalaland of Nigeria

Dickson A. MUSA[1*], Fred O.C. NWODO[2] and Ganiyu O. YUSUF[2]

[1]*Department of Biochemistry, Kogi State University, Anyigba*
[2]*Department of Biochemistry, University of Nigeria, Nsukka*

ABSTRACTS

Some plants used in Igala speaking areas of Kogi State, North-Central Nigeria against a wide range of infectious diseases were studied to determine the basis for the choice of water as the solvent for the extraction of the active components by the natives. The results obtained provide pharmacological evidence to support the use of the plants in the treatment of the diseases caused by the test organisms studied. The result also supports the use of water as the solvent of choice for the extraction of the active components, since the aqueous extracts were found to be more effective than the ethanol and chloroform extracts. The water extracts of all the plant sample were active against Escherichia coli. Eight were active against Salmonella typhi and Staphylococcus aureus. Three of the ethanol extracts were not active against E. coli, S. typhi and five were ineffective on S. aureus. The chloroform extracts did not exert significant antibacterial activity against the three organisms.

Keywords: Igala, Nigeria, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi.*

INTRODUCTION

Medicinal plants have been used for centuries for human diseases because they contain components of therapeutic value. The acceptance now of traditional medicine as alternative form of healthcare and the development of resistance to the available antibiotics have led to widespread investigation of the antimicrobial activity of medicinal plants [1, 2].

Herbal medicine is readily available in our diverse vegetation, cheap and above all carries the potential for introducing new templates into modern medicine [3]. In the Igala speaking areas of Nigeria, herbal medicine practitioners are still consulted as a first choice in the treatment of ailments, due to the fact that traditional medicine blends readily with the socio-cultural life of the people, and the fact that orthodox medicine are more expensive to procure. In addition, orthodox pharmaceutical preparations are many times faked.

There is a vast array of medicinal plants used in the treatments of various ailments in the Igala speaking areas. They are used singly or in combination with other medicinal plants that confer synergistic effect. These medicinal plants or their extracts are administered in different ways depending on the ailment. They could be administered orally, topically, by inhalation of vapours and by steam bath. The medicinal plants are prepared as infusions in hot water, decoction in cold water, concoction with food and as tinctures with alcohol.

The increasing use of plant extracts in the food, cosmetics and pharmaceutical industries suggest that in order to find active compounds, a systematic study of the medicinal plants is important. Findings from various researches suggest that non polar solvents are the best for the extraction of active substances from medicinal plants [4, 5]. Others have suggested that alcohol is the best polar solvent for the extraction of active substances from medicinal plants [6, 7]. In traditional Igala pharmacognosy, the solvents used for extraction are water, ethanol and palm kernel oil, however, it is observed that water is the main extractive solvent used. There is therefore a need to study the comparative antibacterial activity of the aqueous, ethanol and chloroform extracts of some selected medicinal plants used in Igalaland against some bacteria implicated in some of the common diseases prevalent in the area.

The plants used in this study; *Anthocleista vogelii* Planch, *Morinda lucida, Triplochiton scleroxylon, Alchornea cordifolia, Cassia sieberiana, Mangifera indica, Anacardium occidentale, Nauclea latifolia, Daniela oliveri* and *Carica papaya*, are used in Igalaland for the treatment of various ailments including enteric fever, diarrhea, dysentery, malaria, common cold, convulsion, yellow fever, jaundice and dental caries. Some of them are used as poultice on wound or for the treatment of various skin infections.

The microorganisms used in this study; *E. coli*, *S. typhi* and *S. aureus* are known to be causative agent for some of the infections that the people claim to use the plants to treat. *S. typhi* is the causative agent for typhoid fever [8]. *E. coli* is known to be responsible for both intestinal infections such as diarrhea and dysentery, and extra-intestinal infections such as meningitis, peritonitis and septicemia [9, 10, 11]. *S. aureus*, although a normal flora of the skin, should be considered as a potential pathogen [12]. It is an important opportunistic pathogen responsible for a variety of diseases ranging from minor skin infections to life threatening systemic infections such as endocarditis and sepsis.

MATERIALS AND METHODS

The plants were collected from various locations in the Igala speaking areas of North-Central Nigeria. They were identified by Prof. COC Agwu of the Biological sciences Department, Kogi State University, Anyigba, Nigeria. They are as shown in Table 1.

The test organisms: *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* were obtained from the Microbiology Department of Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria.

Preparation of Extracts of Plant Materials

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

For the aqueous extract, 100g of leaf sample were macerated in 750 ml aliquots of distilled water in conical flasks. The mixture was rocked gently to form a homogeneous suspension and

allowed to stand for 24 hours. This was then filtered through Whatman No 1 filter paper using a Speedvac vacuum pump, Buchner funnel and Buchner flask. 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 sample were macerated in 6 volumes (w:v) quantities chloroform-ethanol (2:1) skaken 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. Small quantity of NaCl was added to separate the mixture in 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 (yield = % starting material) and chloroform extracts (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 techniques described by [13] and [14].

0.2ml of the standardized microbial suspension of the test organisms was seeded into 600ml of molten Mueller – Hinton agar at a temperature of 40^0C. The seeded agar was poured aseptically into sterile Petri dishes and allowed to set at room temperature. The solidified agar was bored with sterile 8mm cork borer to create 5 wells on the agar plate about 10mm deep. 0.1ml of 12.5mg/ml, 25mg/ml, 50mg/ml and 100mg/ml of the crude extracts was added to each of the well on the agar plate, the solvent of extraction was also added to the fifth well. The concentrations were achived by reconstituting the crude extracts in their solvents of extraction.

Similarly, 3 plates were prepared for the 3 standard antibiotics; gentamycin, amoxicillin and chloramphenicol, which were used as the control. The plates were incubated for 24 hours at 37^0C. The resulting zones of inhibition in mm were then measured using a transparent ruler. The experiment was carried out in triplicates and the result is recorded as the mean value.

RESULTS AND DISCUSSION

The results obtained suggest that there is a pharmacological basis for the use of some of the plants for the treatment of the diseases. This is because the antibacterial activity of some of the plant extracts compares favourably with those of the standard antibiotics used. The use of water for the extraction of the active agents of the plant materials in Igalaland could have been borne out of experimentation in early days.

Even though Table 2 shows that ethanol had the largest yield, this cannot be related to activity as the aqueous extract was more potent weight for weight, considering the relative sensitivity against each of the bacteria in this study. However, result from Table 3, 4 and 5 suggests that ethanol is a better extractive solvent for the plants whose material used was the bark. The bark of *M. indica* and *A. occidentale* were used against the leaves of the other plants used, and consistently there was a higher antibacterial activity of the ethanol extract compared to the aqueous for all the test organisms used. The mean ethanol yield from *D. oliveri* was 0.01%, whereas the aqueous and chloroform yield were 0.52% and 1.11% respectively, suggesting that ethanol is not a good solvent for the extraction of active components from the plant.

Results obtained show that the aqueous extracts of *A. vogelii* Planch, *M. lucida, T. scleroxylon, A. cordifolia, N. latifolia,* and *C. papaya*, were active against *E. coli* and they had better zone of

inhibitions than those of the ethanol and chloroform extracts. Apart from *T. scleroxylon* whose chloroform extract showed a little activity against *E. coli*, the chloroform extracts of the plants studied did not exhibit any form of activity against *E. coli.* The ethanol extract of two of the plant samples, *M. indica* and *A. occidentale* produced better activity against *E. coli* than their aqueous and chloroform extracts. Notwithstanding, the aqueous extracts of these plants had activity against the test organisms comparable to those of some standard antibiotics like amoxicillin, chloramphenicol and gentamycin. This suggests that water is the best solvent of the extraction for these plants in the treatment of ailments caused by *E. coli*.

Similarly, Table 4 shows that the aqueous extracts of 7 out of the 10 plants studied were active against *S. typhi* with a zone of inhibition higher than those of the ethanol and chloroform extracts, and which are comparable with those of the standard antibiotics used. The aqueous extracts of two plants, *C. sieberiana,* and *M. indica,* were found to be ineffective against *S. typhi* , however, the ethanol and chloroform extracts of these two plants were active against the test organism, with the ethanol extract having the higher activity. For *A. occidentale,* the aqueous and chloroform extracts have comparable antibacterial effect, but these effects were lower when compared with the effects of the ethanol extracts. These findings suggest that alcohol is a good solvent for the extraction of active components from plants used for the treatment of typhoid fever. However, the evidence from Table 4 shows that water is the best extractive solvent of the three solvents.

Table 1: Plant Samples Used

S/N	LOCAL NAME	BOTANICAL NAME	PART USED	LOCATION
1	Odogwu	*Anthocleista vogelii* Planch	leaves	Idah
2	Ugbakolo	*Morinda lucida*	leaves	Anyigba
3	Uwewe	*Triplochiton scleroxylon*	leaves	Ankpa
4	Oyii	*Alchornea cordifolia*	leaves	Ankpa
5	Itolo	*Cassia sieberiana*	leaves	Ofu
6	Umagolo	*Mangifera indica*	bark	Anyigba
7	Opigolo	*Anacardium occidentale*	bark	Anyigba
8	Ogbayi	*Nauclea latifolia*	leaves	Dekina
9	Oda	*Daniela Oliveri*	leaves	Ofu
10	Echibakpa	*Carica papaya*	leaves	Anyigba

Table 2: Comparative Mean Extract Yield

PLANT SAMPLE	AQUEOUS EXTRACT (%)	ETHANOL EXTRACT (%)	CHLOROFORM EXTRACT (%)
Anthocleista vogelii Planch	5.33	6.43	0.77
Morinda lucida	1.55	6.95	1.46
Triplochiton scleroxylon	1.96	0.57	0.53
Alchornea cordifolia	0.67	0.54	0.38
Cassia sieberiana	0.55	0.96	2.20
Mangifera indica	3.75	3.58	1.88
Anacardium occidentale	1.27	3.67	4.78
Nauclea latifolia	2.60	0.56	0.91
Daniela Oliveri	0.52	0.01	1.11
Carica papaya	1.22	0.85	0.88
Mean Yield	1.94±1.6	2.41±2.6	1.49±1.3

The aqueous extracts of *A. occidentale* and *N. latifolia* were found to be ineffective against *S. aureus* whereas their ethanol and chloroform extracts were active. Of note also is the fact that that chloroform extract of *A. occidentale produced* a very high activity against the test organism, which is contrasting to the effects exhibited generally by the chloroform extracts against the test

organisms in the course of this study. However, as shown in Table 5, the aqueous extracts of 8 out of the ten plants studied produced better antibacterial activity against *S. aureus* compared with the ethanol and chloroform extracts.

Table 3: Comparative Sensitivity of *E. coli* to crude extracts at 100mg/ml

	Zone of Inhibition (mm)		
PLANT/CONTROL	**Aqueous extract**	**Ethanol extract**	**Chloroform extract**
Anthocleista vogelii Planch	8	6	0
Morinda lucida	8	0	0
Triplochiton scleroxylon	10	6	2
Alchornea cordifolia	10	3	0
Cassia sieberiana	10	5	0
Mangifera indica	6	10	0
Anacardium occidentale	6	11	0
Nauclea latifolia	10	4	0
Daniela Oliveri	6	0	0
Carica papaya	10	1	0
Amoxicillin	4		
Chloramphenicol	9		
Gentamycin	10		

Table 4: Comparative Sensitivity of *S. typhi* to crude extracts at 100mg/ml

	Zone of Inhibition (mm)		
PLANT/CONTROL	**Aqueous extract**	**Ethanol extract**	**Chloroform extract**
Anthocleista vogelii Planch	10	8	4
Morinda lucida	10	0	6
Triplochiton scleroxylon	8	0	0
Alchornea cordifolia	6	4	0
Cassia sieberiana	0	6	6
Mangifera indica	0	10	4
Anacardium occidentale	6	9	6
Nauclea latifolia	11	4	6
Daniela Oliveri	10	0	0
Carica papaya	9	8	0
Amoxicillin	9		
Chloramphenicol	7		
Gentamycin	17		

Table 5: Comparative Sensitivity of *S. aureus* to crude extracts at 100mg/ml

	Zone of Inhibition (mm)		
PLANT/CONTROL	**Aqueous extract**	**Ethanol extract**	**Chloroform extract**
Anthocleista vogelii Planch	6	0	0
Morinda lucida	10	0	0
Triplochiton scleroxylon	10	3	0
Alchornea cordifolia	10	0	0
Cassia sieberiana	10	7	1
Mangifera indica	6	4	0
Anacardium occidentale	0	4	9
Nauclea latifolia	0	8	2
Daniela Oliveri	8	-	3
Carica papaya	8	0	0
Amoxicillin	7		
Chloramphenicol	17		
Gentamycin	12		

These activities were also comparable with those of standard antibiotics used. This suggests that aqueous extraction is the best form of extraction for plants used against diseases caused by *S. aureus*.

This investigation reveals that there are empirical pharmacological basis for the use of the plant samples by the Igalas for the treatment of a wide range of infective disorders including typhoid fever and wounds. In addition, it ascribes validity to their use of water for the extraction of active components from the samples.

Acknowledgement
The authors are grateful to the following individuals for their technical aid; Ugwu Linda, Tope Opadiran and Erih Moses

REFERENCES

[1]. G.Bisignano, M.P. Germano, A. Nostro, R. Sanogo, *Phytotherapy Research* **1996**, 9, 346.
[2]. K.A. Hammer, C.F. Carson, T.V. Riley, *J. Appl. Microbiol.* **1999**, 86, 985.
[3]. K.O. Akinyemi, S.I. Smith, A.O. Oyefolu, A.O. Coker, *Public Health J. I.* **2005**, 119, 321
[4]. I.I. Ijeh, O.D. Omadamiro, I.J. Nwanna, *Afr. J. Biotechnol.*, **2005**, 4, 9, 953.
[5]. S.A. Junaid, A.O. Olabode, F.C. Onwuliri, A.E.J. Okwori, S.E. Agina, *Afr. J. of Biotechnol.* **2006**, 5, 2315.
[6]. V.I. Obi, C. Onuoha, In J.N. Ogbuile, O.J. Ojiako, (Ed). Biological and Agricultural Techniques. **2000**
[7]. J. Parekh, R. Nair, S. Chanda, *Turk. J. Biol*., **2005**, 31, 58.
[8]. R.A. Gianella, In Baron (Ed) Medical Microbiology 4th Edition. **1996**.
[9]. S. Sela, D. Nestel, R. Pinto, Nemmy-Lavy, M. Bar Joseph, *Appl. Enviro., Microbiol.* **2005**, 71, 7, 4052.
[10]. R. Franiczek, I. Dolna, B. Kryzyanowaska, K. Szufnarowsk, B. Kowalska-Krochmal;, M. Zielinska, *PMID* **2006**, 58, 1, 41.
[11]. M. Girard, D. Steele, C. Chaignat, M. Kieny, *Vaccine* **2006**, 24, 15, 2732.
[12]. R.W. Lacey, *J. Med. Microbiol.* **1972**, 5, 497.
[13]. I.E. Odama, Shok, M. P.F. Olurinola. In A. Sofowora, (Ed.) The State of Medicinal Plant Research in Nigeria. **1986.**
[14]. C. Perez, M. Pauli, P. Berzergue, *Acta Biologiaeet Medicine Experiemtalis;* **1990**, 15, 113.