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Antibacterial activity of the stem barks of *Irvingia wombolu* and *Irvingia gabonensis*

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Abstract
Background and Aim: *Irvingia gabonensis* and *Irvingia wombolu* seeds and stem barks are used in herbal medicine. The stem barks are used against bacterial infections in Nigeria. The present work is aimed at evaluation of the antibacterial activity of the stem barks of *Irvingia gabonensis* and *Irvingia wombolu* grown in Nigeria and also to ascertain which of the *wombolu* and *gabonensis* species has greater antibacterial activity.
Materials and methods: Each of the stem barks was air-dried for one week and ground into powdered form. This was separately extracted with ethanol and water, using Soxhlet extraction apparatus, to give the ethanol and water extracts respectively. Antimicrobial susceptibility of each of these extracts was carried out using agar well diffusion method while minimum inhibitory concentration was determined through broth dilution method.
Results: The test microorganisms, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are susceptible to all the extracts. The *wombolu* species has much stronger antibacterial activity than the *gabonensis* species. The aqueous extract in each case has greater activity than its corresponding ethanol extract.
Conclusion: *Irvingia gabonensis* and *Irvingia wombolu* stem bark extracts have strong antibacterial activity against both gram negative and positive bacteria.

Keywords: *Irvingia gabonensis*, antibacterial activity, *Irvingia wombolu*

1. Introduction
Irvingia gabonensis and *Irvingia wombolu* are mainly known for their seeds used in making *ogbono* soup which is a delicacy in Nigeria [1] The soup is slimy because the seeds are quickly gelatinized on heating with oil and water. This gives a thick slimy stuff made up of polymers of carbohydrates [2, 3, 4].

a *I. wombolu* **b.** *I. gabonensis*

Fig 1: Fruits of *Irvingia species*

a. *I. wombolu,* **b.** *I gabonensis*

Fig 2: *Irvingia* seeds

The fruit of *I. gabonensis* is larger and has edible yellowish pulp with a turpentine flavor when ripe while *I. wombolu* has a smaller fruit with a bitter inedible and acrid pulp (Fig 1) [1]. The *I. wombolu* seeds are smaller in size (Fig 2) but are preferable in soup making because of their greater slimy nature in soup. It also gives stronger flavor to the soup. The seeds of both varieties are commonly mixed by marketers in order to avoid differential prizing by the customers [1]. Most researchers bought their seed samples from the market. Their reports are therefore based on mixed samples. The *I. wombolu* seed has higher protein and ash content while *I. gabonensis* has very high crude lipid content of 74% and lower ash content [1].
Irvingia gabonensis and *Irvingia wombolu* reduce body weight and obesity [5]. They are also used in control of diabetes [6, 7, 8]. The seeds also have wound healing effect [7]. Their stem barks possess hypoglycemic and anti-diabetic properties and also sustained anti-obesity [9]. There is also a claim by some local people in Southern Nigeria that these stem barks control bacterial infections. The present work is therefore aimed at evaluation of the antimicrobial activities of these two species of *Irvingia*.

2. Materials and methods
2.1 Extraction of plant material
The stem barks of *Irvingia wombolu* and *Irvingia gabonensis* were harvested from Asuben forest, Boki local Government Area, Cross River State, Nigeria. The stem barks were air-dried in the laboratory for three weeks and ground. Each of

the ground stem bark (400g) was Soxhlet-extracted with ethanol and another 400g was extracted with water and concentrated to give ethanol extracts and aqueous extracts of both species.

2.2 Antimicrobial susceptibility test

Five different concentrations of each of the four extracts containing 400, 200, 100, 50 and 25mgdm^{-3} were prepared. The test organisms used are *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumonia*. These are clinical isolates obtained from the Pathology Laboratory, University of Calabar Teaching Hospital.

A sterile swab was dipped into the appropriate test organisms, suspended in normal saline, was used to uniformly seed previously prepared Mueller Hinton agar plates. Sterilized cork-borer (5mm diameter) was used to make the wells on the agar plates. The different solutions of the extract were used to fill each well using sterilized Pasteur pipette. The plates were incubated at 37^{0}C for 18h. The diameter of the zone of inhibition around each well was measured and used to determine the microbial susceptibility of each extract at different doses [10, 11]

2.3 Minimum inhibitory concentration

For determination of minimal inhibitory concentration (MIC) 50, 25, 12.5, 6.25, 3.13 and 1.75 mgdm^{-3} of each extract was placed in different test tubes and 1cm^3 of hexane added to each of them. 4cm^3 of peptone water (Mueller Hinton broth was added followed by addition of 4cm^3 of 24h –broth culture of the microorganism. The test tubes were all sealed with sterile corks and subsequently incubated at 37^{0}C for 48h. After incubation the test tubes were observed for clearance or turbidity. The test tube with highest degree of clearance was taken as the MIC while the tube preceding the MIC tube is regarded as the minimum bactericidal concentration (MBC).

3. Results

Table 1: Antimicrobial activity of ethanol extracts of *Irvingia wombolu* and *Irvingia gabonensis* (zone of inhibition, mm).

Concentration/organism	400mgdm^{-3}		200mgdm^{-3}		100mgdm^{-3}		50mgdm^{-3}		25mgdm^{-3}	
	W	G	W	G	W	G	W	G	W	G
E. coli	42	39	38	34	32	30	28	25	23	21
S. aureus	39	37	34	33	29	28	25	24	21	20
P.aeruginosa	38	35	33	30	28	26	23	21	20	17
K. pneumonia	47	36	41	33	35	30	34	19	30	25

Table 2: Antimicrobial activity of aqueous extracts of *Irvingia wombolu* and *Irvingia gabonensis* (zone of inhibition, mm)

Concentration/organism	400mgdm^{-3}		200mgdm^{-3}		100mgdm^{-3}		50mgdm^{-3}		25mgdm^{-3}	
	W	G	W	G	W	G	W	G	W	G
E. coli	47	39	41	35	34	30	30	28	27	21
S. aureus	40	37	38	33	30	28	28	24	23	20
P. aeruginosa	43	35	32	30	29	27	25	22	22	18
K. pneumonia	41	38	39	33	36	30	31	26	29	21

***Key*:** W =*I. wombolu* extract, G = *I. gabonensis* extract

Table 3: MIC of ethanol and water extracts of *Irvingia gabonensis* and *Irvingia wombolu* against some micro-organisms.

samples(mm)/micro-organisms	A(mgdm^3)	B(mgdm^{-3})	C(mgdm^{-3})	D(mgdm^{-3})
E. coli	27	30	32	34
Staphylococcus aureus	28	29	29	30
Pseudomonas aeruginosa	27	26	28	29
Klebsiella pneumonia	30	30	35	36

Key: Sample A = ethanol extract of *Irvingia gabonensis*
Sample B =water extract of *Irvingia gabonensis*
Sample C = ethanol extract of *Irvingia wombolu*
Sample D = water extract of *Irvingia wombolu*

The results of antimicrobial susceptibility test for ethanol extracts of *Irvingia wombolu* and *Irvingia gabonensis* are given in Table 1 while Table 2 shows those of the aqueous extracts. The minimum inhibitory concentrations (MIC) of the four extracts are shown in Table 3. The work shows that the four extracts have activity against the entire test organisms and the activity is dose dependent.

4. Discussion

The aqueous extracts of *I. wombolu* and *I. gabonensis* inhibited the growth of gram-negative *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeurginosa* as well as the gram-positive *Staphylococcus aureus.* Their antibacteial activity is dose dependent as it increases with an increase in the level of each of the four extracts with the *wombolu* species

having greater activity. Similarly the antibacterial activities of the ethanol extracts are also dose dependent with the *wombolu* species being more active. From Tables 1, 2 and 3, it is clear that the *wombolu* stem bark has stronger antibacterial activity as it has larger zone of inhibition in both ethanol and aqueous extract at the different levels than the *gabonensis* extracts. In both plants, the aqueous extracts of the stem barks exhibited stronger antibacterial activities than the ethanol extracts. This is attributable to the higher solubility of the active principles in the more polar water solvent. This implies that the antibacterial principles of the *Irvingia gabonensis* and *Irvingia wombolu* are polar compounds. All the test microorganisms were susceptible to the four extracts at the different levels used. This is in accord with the result of the minimum inhibitory concentrations recorded in Table 3. With increase in resistance of pathogenic microorganisms to the conventional antibiotics, the *Irvingia gabonensis* sand *Irvingia wombolu* stem barks could serve as a suitable alternative to the synthetic drugs used in orthodox medicine. It also has the added advantage of being cheap and environmentally friendly [12].

Conclusion

The two stem barks showed strong antibacterial activities against the test microorganisms. The *wombolu* stem bark is more efficacious than the *gabonensis* stem bark. The present work therefore justifies their use in herbal medicine for control of bacterial infections.

Conflict of interest

The authors declare no conflict of interest.

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