



## Phytochemical and Antibacterial Evaluations of Chloroform Extract of *Mondia whitei* (Hook F) Skeels

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### Abstract

*Mondia whitei*, also known as *Mondia*, is an aromatic plant of Apocynaceae family. It is used as aphrodisiac, antidepressant, fertility medication and to improve appetite. The present study aimed to evaluate the phytochemical and antibacterial properties of the plant. The chloroform extract of *M. whitei* was screened for its antibacterial effects using agar diffusion method against five bacterial strains (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi*). The extract was active against *S. typhi*, *K. pneumoniae* and *S. aureus* at 400, 400 and 200 mg/mL, respectively. The antibacterial activity of the extract was observed in a dose-dependent manner and was compared with standard antibiotic, ciprofloxacin. The phytochemical screening of the extract revealed the presence of flavonoids, terpenoids, cardiac glycosides, steroids, phlobatannins and phytosterols. The GC-MS analysis of the extract afforded seventeen (17) compounds with major components being n-hexadecanoic acid (23.80%). The exhibited antibacterial activities justify the ethnomedicinal uses of *M. whitei* root.

**Keywords:** *Mondia whitei*, GC-MS, Antibacterial activity, Phytochemical

### Introduction

Medicinal plants have been used for the treatments of many infectious diseases without any scientific evidence. At present, there is more emphasis on determining the scientific evidence and rationalization for the use of these herbal preparations (Thompson 2006). According to the World Health Organisation, 80% of the world inhabitants depend on traditional medicines for their basic health requirements. The vast majority of these medicines are obtained from plants as complementary or alternative medicines (Farnsworth et al. 1985).

Antimicrobial crucial part is to protect against damage caused by microorganisms.

Microbial infections have always been the major contributors to the development of many diseases such as sexually transmitted diseases (Vermani and Garg 2002). Herbal preparations have been shown to reduce the risks of the diseases caused by microorganisms (Neto et al. 2002). Microorganisms cause many diseases such as meningitis (*Neisseria meningitidis*), tuberculosis (*Mycobacterium tuberculosis*), pneumonia (*Streptococcus pneumoniae*), gastritis (*Helicobacter pylori*), and typhoid fever (*Salmonella typhi*). In previous years, many of these organisms have shown rapid resistance against current antimicrobial agents (Gupta et al. 2019); hence, there is an

urgent need for alternative therapies for the treatments of the diseases caused by these microbes.

*Mondia whitei* (Hook F.) skeels, also known as African ginger, white ginger or simply as *Mondia* belong to the family Apocynaceae. It is a perennial climbing shrub with a large tuberous root stock. The root is aromatic and tastes like ginger or liquorice and has an aroma of vanilla (Iwu 2014). Most ethnic groups use *M. whitei* to treat gastrointestinal conditions such as mild laxative, appetite stimulant, alleviation of pain and nausea (Aremu et al. 2011). It is used in the treatment of headache, jaundice, diarrhoea, impotence and urinary tract infections (Watcho et al. 2007). Martey and He (2010) reported that Ghana traditional practitioners used *Mondia* in the management of erectile dysfunction and low sperm counts. The plant root is sometimes consumed as food and as herbal tea and flavours (Gericke 2000, McGeoch 2004).

Kubo and Kinst-Hori (1999) reported the isolation of 2-hydroxy-4-methoxybenzaldehyde from *M. whitei*. Koorbanally et al. (2000) reported the isolation of vanillin from the root of *M. whitei*. Iwu (2014) reported the nutritional content of *M. whitei* which is very rich in vitamins and minerals.

*M. whitei* has been reported to exhibit a broad spectrum of pharmacological properties and possesses aphrodisiac (Lampiao 2009, Ngbolua et al. 2018), antibacterial (Gbadamosi and Erinoso 2015) and anti-diarrhoeal activities (Ndukupi et al. 2013). However, information on the chemical constituents and antibacterial activities of the root of *M. whitei* is grossly inadequate. Furthermore, the assessment of the ethnomedicinal properties of the plant remains an interesting task to find new promising sources of natural antibacterial agents for functional food and dietary supplements. Therefore, it is worthwhile to investigate the chemical constituents and antibacterial activities of the root of *M. whitei*. The objective of this study was to identify and examine the antibacterial activities and composition of *M. whitei* root.

## Materials and Methods

### Sample preparation

The roots of *M. whitei* were collected from Lokoja, Kogi State, Nigeria. The roots were cut into pieces using a knife and air dried at room temperature. The dried sample was pulverized using a wooden mortar and pestle. The pulverized sample was poured into an extraction bottle and 1000 mL of analytical grade chloroform was added. It was allowed to extract for seven days at room temperature and was filtered with Whatman filter paper. The filtrate was poured into an evaporating dish and left to dry at room temperature to obtain the chloroform crude extract.

### Phytochemical analysis of the chloroform fraction of *M. whitei* root

Preliminary phytochemical analysis of the chloroform fraction was carried out using the method described by Jones and Kinghorn (2006).

### Characterization of the extract and fractions of *M. whitei* root using GC-MS

Gas chromatography-mass spectrometry analysis of chloroform extract of *M. whitei* root was performed using GCMS-QP2010 Plus Shimadzu, Japan. The extract was introduced and volatilized in the injection part of the gas chromatograph. Compounds were separated in HP5MS column fused with phenylmethylsilox (length; 30 m × 250 µm; film thickness 0.25 µm). Sample was injected at a temperature of about 250 °C with a split ratio of 10:1 with a flow rate of helium 1 mL/min. The compounds were identified by comparing the similarity of the mass spectra and retention times with the standard solutions and with the literature.

### Media

Pure culture of the isolates used in this study were obtained from Ahmadu Bello University Teaching Hospital, Shika, Kaduna State, Nigeria and preserved in McCartney bottles with slant preparation of nutrients agar to maintain their growth. The test culture consisted of four Gram-negative bacteria: *Escherichia coli*, *Klebsiella pneumoniae*,

*Pseudomonas aeruginosa*, *Salmonella typhi* and one gram-positive bacteria; *Staphylococcus aureus*. Nutrient broth (OXOID) was used and chloroform was used as negative control in the assay.

### Antibacterial assay

Antibacterial activity of the chloroform extract was tested using the paper disc diffusion method described by Rahman et al. (2007). Sterile 6 mm disc Whatman number 1 filter paper disc was impregnated with varying concentrations of the extract 400, 200, 100 and 50 mg/mL. The bacterial cultures were inoculated on nutrient broth (OXOID) and incubated for 24 h at 37 °C. Adequate amounts of nutrient agar (OXOID) were dispensed into sterile plates and allowed to solidify under aseptic conditions. The bacterial cultures were adjusted to match McFarland turbidity standard. The test microorganisms were inoculated with a sterile swab on the surface of appropriate solid medium in plates. The agar plates inoculated with the test microorganisms were incubated for 1 hour before placing the extract impregnated paper disc on the plates. The bacterial plates were incubated at 37 °C for 24 h, all plates were observed for zones of growth inhibition and the diameters of these zones were measured in millimeters. All tests were performed under sterile conditions.

### Statistical analysis

The results were expressed as mean  $\pm$  SD of triplicate samples. Statistical analysis was performed by one-way ANOVA using SPSS version 20. The obtained results were considered statistically significant at  $p \leq 0.05$ .

## Results and Discussion

### Phytochemical content of the chloroform extracts of *M. whitei* root

The phytochemical analysis of the chloroform extract of *M. whitei* root revealed the presence of flavonoids, terpenoid, cardiac glycosides, steroids, phlobatannins and phytosterols, as shown in Table 1. The presence of phytosterols terpenoids and flavonoids makes the root an important source of food and flavouring agents

(Gericke 2000, Koorbanally et al. 2000, Mcgeoch 2004, Iwu 2014).

**Table 1:** Phytochemical content of chloroform extract of the root of *M. whitei*

Phytochemical	Chloroform fraction
Flavonoids	+
Terpenoids	++
Cardiac glycoside	+
Tannins	-
Steroids	+
Saponins	-
Phlobatannins	+
Phytosterols	+
Alkaloids	-

Key: - = not detected, + = trace amount, ++ = moderate amount. Flavonoid, terpenoids, cardiac glycoside, steroids, phlobatannins and phytosterol were detected, while tannins, saponins and alkaloids were absent.

### GC-MS results of the chloroform extract of *M. whitei* roots

GC-MS analysis of *M. whitei* root revealed the presence of 17 compounds with major compounds as n-hexadecanoic acid (23.80%), trans-carvone oxide (12.36%) and other minor compounds as indicated in Table 2.

### Antimicrobial activities of *M. whitei* root

The outcomes of the antimicrobial activities of the plant extract are shown in Figures 1-2 and Tables 3-4. The antimicrobial activity of the extract was tested at different concentrations: 400 mg/mL, 200 mg/mL, 100 mg/mL and 50 mg/mL. These were compared with antimicrobial activity of standard drug, ciprofloxacin (5  $\mu$ g/ mL). The extract showed antimicrobial activities against the growth of *Klebsiella pneumoniae* and *Staphylococcus aureus* at the concentrations of 400 mg/mL to 200 mg/mL, *Pseudomonas aeruginosa* at the concentration of 400 mg/mL and *Salmonella typhi* at the concentrations of 400 mg/mL to 100 mg/mL. The extract had no activity against *Escherichia coli* even at the highest concentration (400 mg/mL) used for this study. This result lend support to the earlier report by Gbadamosi and Erinoso (2015) which confirmed the activity of the aqueous

root extract of *M. whitei* against the four organisms. The inhibitory effect of the extract is concentration dependent as illustrated in Figures 1 and 2. Higher concentrations show

higher effects. The inhibitory effect disappeared completely at a lower concentration of 50 mg across all the microorganisms.

**Table 2:** Compounds identified in GC-MS analysis of chloroform extract of *M. whitei*

S/N	Compound	Peak area (%)	Molecular weight (g/mol)	Retention time
1	4-Penten-2-one,4-methyl	0.47	98	5.625
2	1,4-Butanediol, diacetate	1.11	174	5.800
3	2-Pentanol, acetate	0.50	130	6.767
4	5H-1-Pyridine	1.06	117	8.633
5	Camphenone	1.56	150	11.000
6	Phenol,3,5-bis(1,1-dimethylethyl)	2.32	206	11.258
7	Acetic acid, octyl ester	1.86	172	11.617
8	(1R,2R,3S,5R) (-)-2,3-Pinane diol	4.12	170	12.150
9	3-Cyclopentene-1-acetaldehyde,2,2,3-trimethyl	6.93	152	12.917
10	7-Oxabicyclo [4.1.0] heptane, 1-methyl-4-(2-methyloxiranyl)	7.14	168	13.175
11	Carvone oxide, trans	12.36	166	15.033
12	Triacitanoic acid, methyl ester	1.66	368	16.842
13	n-Hexadecanoic acid	23.80	256	17.958
14	9,12-octadecadienoic acid, methyl ester	2.02	294	19.875
15	Phytol	3.99	296	20.208
16	Nonadecanoic acid	7.42	298	21.008
17	9-Octadecenamamide, (Z)	8.75	281	23.267

**Table 3:** Antimicrobial activities of chloroform extract of *M. whitei* root on selected bacteria

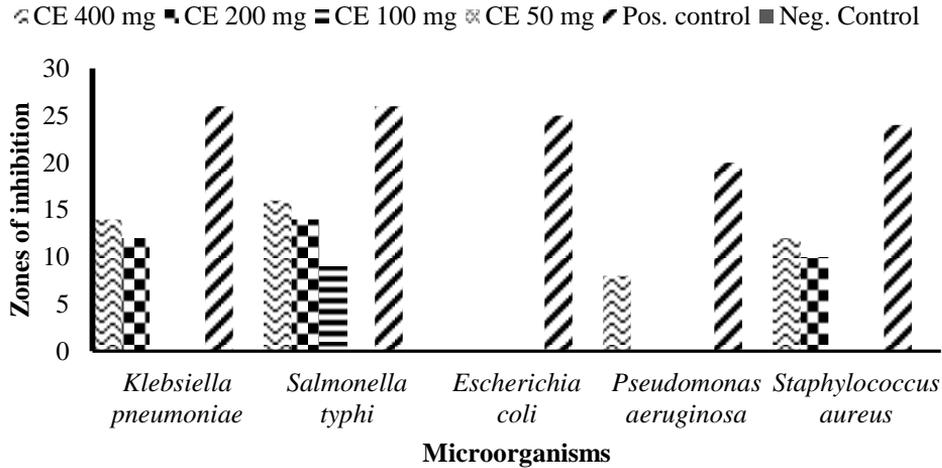
Microorganisms	Concentration of fraction					
	400	200	100	50	+ve	-ve
	Zone of inhibition (mm)					
<i>K. pneumoniae</i>	14	12	0	0	26	0
<i>E. coli</i>	R	0	0	0	25	0
<i>S. aureus</i>	12	10	0	0	24	0
<i>P. aeruginosa</i>	8	R	0	0	20	0
<i>S. typhi</i>	16	14	9	0	26	0

Key: Positive control (+ve) (ciprofloxacin) = 5 µg/mL, Negative control (-ve) (chloroform), and R = Reactive.

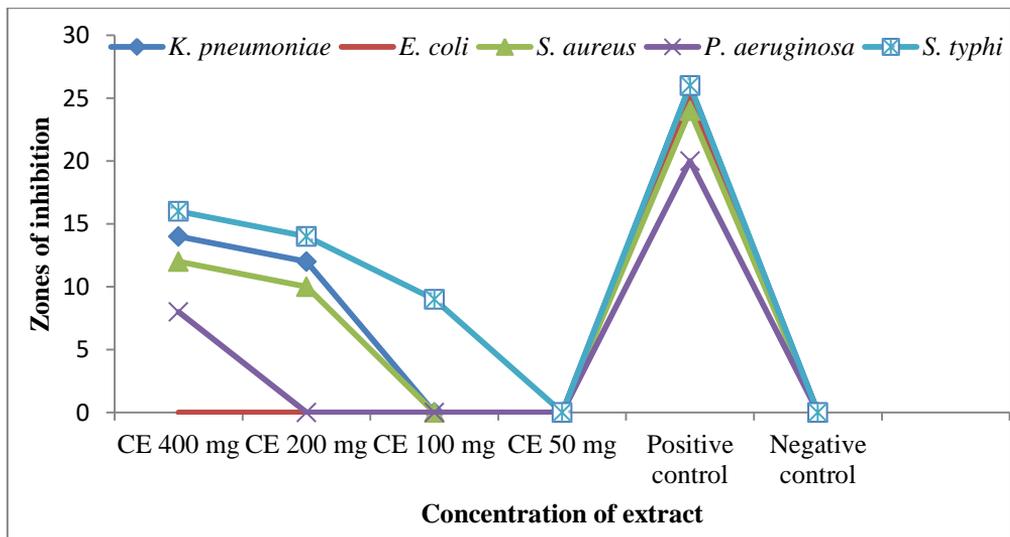
**Table 4:** Effects of chloroform extract of *M. whitei* on zones of inhibition in different microorganisms

Extracts	<i>Klebsiella pneumoniae</i>	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
CE 400 mg	14.00 <sup>abc</sup> ± 1.00	16.00 <sup>abc</sup> ± 1.00	N	8.00 <sup>ab</sup> ± 1.00	12.00 <sup>abc</sup> ± 1.00
CE 200 mg	12.00 <sup>ab</sup> ± 0.00	14.00 <sup>ab</sup> ± 1.00	N	N	10.00 <sup>ab</sup> ± 0.00
CE 100 mg	0.00	9.00 <sup>a</sup> ± 1.00	N	N	N
CE 50 mg	N	N	N	N	N
Pos. control	26.00 <sup>a</sup> ± 1.00	26.00 <sup>a</sup> ± 1.00	25.00 <sup>a</sup> ± 1.00	20.00 <sup>a</sup> ± 1.00	24.00 <sup>a</sup> ± 1.00
Neg. Control	N	N	N	N	N

CE = chloroform extract, N = no zone of inhibition was found. Lower case letters across each column for the extract indicate significant difference ( $p < 0.05$ ). a = significantly higher than negative control, b = significantly lower than the positive control. C = significantly higher than corresponding extract at 200 mg.



**Figure 1:** Activities of chloroform extract of *M. whitei* on zones of inhibition of different microorganisms.



**Figure 2:** Activities of chloroform extract of *M. whitei* on zones of inhibition of different microorganisms.

**Conclusion**

The root of *M. whitei* root revealed that it contains phytochemicals of medicinal importance. The antimicrobial potentials of the chloroform extract of *M. whitei* were determined on five human pathogenic bacteria and the extract exhibited significant zones of inhibition across all the organisms except on *Escherichia coli* which was inhibited only by the positive control. Chloroform extract inhibits the growth of

*Salmonella typhi* at 400 mg, 200 mg and 100 mg, while 50 mg shows no inhibition on the organism. *Pseudomonas aeruginosa* was inhibited by chloroform extract at only 400 mg, which further suggests that the inhibition is concentration-dependent. It is evident from the present study that the phytochemical and biological investigations of the root of *M. whitei* have provided scientific evidence for the ethnotherapeutic claims.

## Declaration of Interest

The authors have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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