



Phytochemical Screening and *in vitro* Antimicrobial Activity of *Waltheria indica* Linn Root Extracts

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Abstract: The aim of this study was to examine the phytochemical constituents and evaluate the antimicrobial activity of *Waltheria indica* Linn root extracts in solvents of different polarities (ethyl acetate, chloroform and chloroform: methanol (1:1)). The experiment was conducted at the Chemistry Advanced Research Centre of Sheda Science and Technology Complex, Abuja, Nigeria between November 2016 and January 2017. Clinical strains of five bacteria and three fungi isolates were utilized. Chloroform, ethyl acetate and chloroform: methanol (1:1) root extracts were tested at 12.5, 25.0 and 50.0 µg/ml using the Agar well diffusion technique. The minimum inhibitory, minimum bactericidal and minimum fungicidal concentrations of each solvent extract were assessed. Phytochemical analysis was also performed using ethanol as the extraction solvent. The phytochemical compounds obtained in the methanol extracts were alkaloids, cardiac glycosides, flavanoids, phenolic acids, saponins, steroids, tannins and terpenoids. Agar well diffusion used for sensitivity study of *S. aureus*, *S. pneumonia*, *S. pyrogens*, *K. pneumonia*, *C. ulcerans*, *C. albicans*, *C. krusei* and *C. tropicalis* with 10µg/ml ciprofloxacin and 30µg/ml fluconazole revealed that the ethyl acetate extract of *Waltheria indica* Linn gave 27±0.20 to 31±0.95mm zones of inhibition, whereas the chloroform extract gave an inhibitory range of 26±0.15 to 30±0.25mm while the chloroform: methanol extracts gave a 26±0.35 to 29±0.85mm zones of inhibition respectively in comparison to the 31±0.65 to 36±0.35mm obtained from the control. The minimal inhibitory content (MIC) of the ethyl acetate extracts was recorded at 0.25 mg/ml, while the MIC values for the chloroform and chloroform: methanol extracts were between 0.25 and 0.50 mg/ml respectively. The results obtained suggested that the studied plants possess anti-microbial spectrum aligned to the phyto-constituents.

Keywords: Phytochemicals, Anti-Microbial Activity, *Waltheria indica* Linn, Therapeutic

1. Introduction

Plants indigenous to West African possess a rich array of bioactive compounds, useful for the treatment of a variety of diseases that are unique to the region [1]. Traditional medicine, practiced across the African continent, utilize a large number of plants whose diversity creates an arsenal of multifunctional phyto-compounds. These phyto-compounds serve as essential raw materials for new drug discovery whereby they possess the inherent advantage of serving as a cheaper, readily available therapeutic agent. For example, *Artemisia annua*, indigenous to the African region, was

discovered to be active against *Plasmodium falciparum* through a series of *in vitro* experiments, the result of which led to the isolation of artemisinin towards the treatment of malaria [2]. Other plants like *Lunasia amara* and *Solanum sodomaeum* were found to be active against *Mycobacterium tuberculosis*, thus prompting the isolation of Graveolinine and Solsodominine respectively for the treatment of tuberculosis [3].

Nigeria is one of several countries in the African country that possesses a rich diversity of untapped botanical resources which could be applied to the treatment of many diseases prone to the country [1, 4]. One of such plants is *Waltheria indica* Linn (*hankufah* in Hausa, *korikodi* in

Yoruba), widely distributed across the South Western and North Central parts of Nigeria. Traditional uses of the plant includes the treatment of diarrhea, stomach ache, wounds, cough, haemorrhage, fever, malaria, skin diseases and can be consumed as an aphrodisiac [6, 7]. With the known uses of the plant, it is possible that *Waltheria indica* Linn can be utilized in the treatment of other medical conditions brought about by pathogenic organisms. Diseases causing pathogens that have commonly been isolated from community and hospital acquired upper respiratory tract infections include *Staphylococcus aureus*, *Streptococcus pneumonia*, *Klebsciella pneumonia*, *Candida spp.*, etc [8]. As part of our research efforts on Nigerian medicinal plant parts, this study was conducted to examine the phytochemical constituents and determine the antimicrobial activity of the roots of *Waltheria indica* Linn.

2. Materials and Methods

The roots of *Waltheria indica* Linn were collected in fresh condition from Kwali (8°50'49"N 7°3'38"E) region of Abuja suburbs in the F. C. T., Nigeria. The plant was authenticated and deposited in the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja where a voucher specimen (No.6738) was given. The collected root was cut into small pieces and air-dried. The dried pieces were pulverized, sieved and stored in a well-ventilated area. 1kg of the plant sample was exhaustively extracted using a soxhlet extractor. The marc was extracted successfully using 2.5L each of ethyl acetate, chloroform and chloroform: methanol (ratio 1: 1). The crude extracts were later concentrated to a minimum volume using rotary evaporator (Büchi Labortechnik AG, Switzerland) at 40°C and reduced pressure. They samples were then kept in an airtight container until required for further laboratory analysis.

2.1. Phytochemical Screening

Phytochemical screening was carried out on the ethyl acetate, chloroform and chloroform: methanol extracts for the qualitative determination of major constituents using methods previously described [9].

2.2. Antimicrobial Screening

Bacterial and fungal isolates were collected from the Department of Medical Microbiology, Ahmadu Bello University Teaching hospital, Zaria, Kaduna State, Nigeria. The bacteria were confirmed using standard biochemical tests according to the Bergey's manual of Bacteriology, while the fungal isolates were identified using fungi chrome test kits [10]. Agar diffusion method was adopted [11].

2.3. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined on the test organisms that were sensitive to the extracts and was done by broth dilution method [12]. Two

fold serial dilution of the extract in sterilized broth was made to obtain the concentration of 50.0 µg/ml, 25.0 µg/ml and 12.5 µg/ml. The initial concentration was obtained by dissolving 6.0 mg of the extract in 10mls of sterile broth. Having obtained the different concentration of the extracts in the sterile broth, was observed for turbidity (growth), the lowest concentration of the extract in the broth which shows no turbidity was recorded to the MIC.

2.4. Determination of Minimum Bactericidal and Fungicidal Concentrations

Minimum bactericidal concentration (MBC) and Minimum fungal concentration (MFC) were evaluated by plating the bacterial suspensions from individual well at the beginning and at the end of the experiments on Mueller Hinton agar medium for estimation of MBC [12]. The culture from MIC well was taken and streaked on the surface of fresh Mueller Hinton agar in a 90-mm plate with division and incubated at 37°C for 24 hours (bacteria) and 30°C for 1-7 days (fungi) after which the plates of the medium was observed for colony growth, the MBC/MFC were the plates with lowest concentration of the extract without colony growth.

3. Results

Table 1 represents the phytochemical composition of ethanol extracts from the roots of *Waltheria indica* Linn. Compounds detected included alkaloids, cardiac glycosides, flavonoids, phenolic acids, saponins, steroids, tannins and terpenoids. The action of these compounds working in concert would enhance their defensive properties against a host of pathogenic organisms by way of eliciting anti-oxidative processes that thwarts pathogen-induced free radicals [29]. The detected compounds were also indicative of their polarity.

Table 1. Phytochemical screening of *Waltheria indica* Linn crude root extract.

Phyto-constituents	Root extract
Tannins	+
Saponins	+
Flavonoids	+
Steroids	+
Phenolic acid	+
Alkaloids	+
Cardiac glycosides	+
Terpenoids	+
Carbohydrates	+

(+) – Present (-) – Absent.

The mean zones of inhibition of the crude extract against 5 bacterial and 3 fungal species are summarized in Table 2. The ethanol extract of *Waltheria indica* Linn root were found more active against *Candida krusei* with 26.0 ± 0.70 mm zone of inhibition followed by *Corynebacterium ulcerans* (25.0 ± 0.35 mm) and *Streptococcus pneumonia* (25.0 ± 0.12 mm) respectively, while the lowest value 23.0 ± 1.10 mm was recorded for *Candida tropicalis*. Ciproflaxin was employed

as the drug control standard for bacteria with a zone of inhibition value ranging from 30 to 35 mm, while

fluconazole was used as the active antifungal drug control which gave a zone of inhibition ranging from 34 to 35 mm.

Table 2. Antimicrobial activity of *Waltheria indica* Linn crude extracts against test microorganisms (mm).

Microorganisms	Crude Root	Control 1	Control 2
<i>Staphylococcus aureus</i>	24 ± 0.65	0	32 ± 0.45
<i>Streptococcus pneumonia</i>	25 ± 1.20	0	30 ± 0.25
<i>Streptococcus pyrogenes</i>	0	0	32 ± 0.30
<i>Klebsiella pneumonia</i>	24 ± 0.90	0	35 ± 0.10
<i>Corynebacterium ulcerans</i>	25 ± 0.35	0	0
<i>Candida albicans</i>	0	35 ± 0.95	0
<i>Candida krusei</i>	26 ± 0.70	35 ± 0.55	0
<i>Candida tropicalis</i>	23 ± 1.10	34 ± 0.60	0

Control 1= Fluconazole (30µg/ml), Control 2= Ciprofloxacin (10µg/ml).

The mean zones of inhibition of solvent specific root extracts against 8 different microorganisms are depicted in Table 3. The ethyl acetate extract of *Waltheria indica* Linn root were found to be more active against *Candida krusei* with 31.0 ± 0.95mm zone of inhibition, *Candida tropicalis* and *Klebsiella pneumonia* with 30.0 ± 0.25mm and 30.0 ± 0.15mm zone of inhibition followed by 29.0 ± 0.45mm against *Corynebacterium ulcerans*, while the lowest value 27.0 ± 0.20 mm was recorded for *Streptococcus pneumonia*. The chloroform extract showed the highest value of 30.0 ± 0.25 mm against *Candida krusei* followed by 29.0 ± 0.85 mm

against *Klebsiella pneumonia* while 26.0 ± 0.15 mm zone of inhibition was noted against *Staphylococcus aureus*.

Using chloroform: methanol, the root extracts were most resistant to *Klebsiella pneumonia* with a zone of inhibition of 29.0 ± 0.85 mm whereas the least zone of inhibition of 26.0 ± 0.35 mm was observed against *Candida tropicalis*. The data indicated that the root extract of *Waltheria indica* Linn was sensitive towards *Streptococcus pyrogenes* and *Candida albicans* as no zone of inhibition was observed using all three solvent extracts.

Table 3. Zone of inhibition of the root fractions against the test microorganism.

Test microorganisms	Ethyl Acetate	Chloroform	Chloroform: Methanol (1: 1)	Control
<i>Staphylococcus aureus</i>	28 ± 0.75	26 ± 0.15	28 ± 0.25	32 ± 0.15 (C)
<i>Streptococcus pneumonia</i>	27 ± 0.20	28 ± 0.90	26 ± 0.90	31 ± 0.65 (C)
<i>Streptococcus pyrogenes</i>	0	0	0	32 ± 0.75 (C)
<i>Klebsiella pneumonia</i>	30 ± 0.15	29 ± 0.85	29 ± 0.85	34 ± 0.80 (C)
<i>Corynebacterium ulcerans</i>	29 ± 0.45	28 ± 1.05	27 ± 0.40	0 (C)
<i>Candida albicans</i>	0	0	0	36 ± 0.35 (F)
<i>Candida Krusei</i>	31 ± 0.95	30 ± 0.25	28 ± 0.10	34 ± 0.90 (F)
<i>Candida tropicalis</i>	30 ± 0.25	27 ± 0.10	26 ± 0.35	33 ± 0.10 (F)

C = Ciprofloxacin (10µg/ml), F = Fluconazole (30µg/ml).

Table 4 data shows the minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and the minimal fungicidal concentration (MFC) of the different extracts of *Waltheria indica* Linn. The ethyl acetate extracts possessed the lowest MIC value (0.25 mg/ml) against all tested clinical organisms. This was the same for all but against *Streptococcus aureus* which depicted an MIC value of 0.5 (mg/ml) similar to the MIC values obtained against

Streptococcus pneumonia using the chloroform: methanol extracts. The MIC values for the crude extract was 2.5 (mg/ml) against all tested microorganisms (Table 4). The MBC and MFC values were roughly the same for the extracts with the exception of 1.0 mg/ml recorded against *Streptococcus pneumonia* for the ethyl acetate extracts (Table 5). The MBC and MFC values of the crude extract was 5.0 (mg/ml) across all tested clinical isolates.

Table 4. Minimum inhibitory concentration (MIC) of *Waltheria indica* Linn root extracts against test organisms Zone of inhibition (mg/ml ± SD).

Microorganisms	Minimum Inhibitory Concentration (mg/ml)			
	Crude	Ethyl Acetate	Chloroform	Chloroform: Methanol (1: 1)
<i>Staphylococcus aureus</i>	2.5	0.25	0.5	0.25
<i>Streptococcus pneumonia</i>	2.5	0.25	0.25	0.5
<i>Streptococcus pyrogenes</i>	-	-	-	-
<i>Klebsiella pneumonia</i>	2.5	0.25	0.25	0.25
<i>Corynebacterium ulcerans</i>	2.5	0.25	0.25	0.25
<i>Candida albicans</i>	-	-	-	-
<i>Candida krusei</i>	2.5	0.25	0.25	0.25
<i>Candida tropicalis</i>	2.5	0.25	0.25	0.5

Table 5. Minimum bactericidal and fungicidal concentrations (MBC, MFC) of *Waltheria indica* Linn root extracts against test organisms (mg/ml \pm SD).

Microorganisms	Minimum Bacterial/Fungal Concentration (mg/ml)			
	Crude	Ethyl Acetate	Chloroform	Chloroform: Methanol (1: 1)
<i>Staphylococcus aureus</i>	5.0	0.5	1.0	0.5
<i>Streptococcus pneumonia</i>	5.0	1.0	0.5	1.0
<i>Streptococcus pyogenes</i>	-	-	-	-
<i>Klebsiella pneumonia</i>	5.0	0.5	0.5	0.5
<i>Corynebacterium ulcerans</i>	5.0	0.5	0.5	1.0
<i>Candida albicans</i>	-	-	-	-
<i>Candida krusei</i>	5.0	0.5	0.5	0.5
<i>Candida tropicalis</i>	5.0	0.5	1.0	1.0

Each value represents mean (n = 3).

4. Discussion

In this investigation, the discovery of particular secondary metabolites proffers the initial scientific insight into the therapeutic potential of the plant against diseased conditions emanating from a host of pathogens [13, 14]. Findings from table 1 suggest that the roots contain a number of secondary metabolites, namely; alkaloids, cardiac glycosides, flavonoids, phenols, saponins, steroids, tannins and terpenoids in all solvent extracts are known individually or collectively for their defensive or signaling functions [15]. Alkaloids are useful neuro-stimulatory agents that also possess analgesic and upper respiratory protective functions amongst others [3, 16, 17, 18]. Steroids enhances the immune system by preventing penetration of many pathogens owing to their antimicrobial properties [13]. The presence of both Alkaloids and Steroids in the root extracts suggests that this part of the plant possesses sufficient activity against disease causing pathogens like *S. aureus* and *S. typhi* which are responsible for diarrhea and stomach upsets [19]. Phenolic acids have been reported to exhibit anti-allergic, anti-inflammatory, antioxidant, antimicrobial, anticancer and antidiarrheal properties [20, 21]. Their presence in the plant extracts suggest a strong and wide range of therapeutic applications whereby they thwart the deleterious effects of disease-causing pathogens by inhibiting their growth and proliferation. The mean zones of inhibition of the crude extract using disc diffusion method against different bacterial and fungal species revealed a high antimicrobial activity against selected pathogenic organisms implicated in several upper respiratory tract infections (Table 2). Notably, against *Corynebacterium ulcerans* (25 mm) in comparison with the control; Ciprofloxacin (10 μ g/ml), the data obtained suggests the possibility for isolating an alternative or new drug candidate from this plant. The antifungal activity was high against *Candida tropicalis* (24mm) as the plant extracts displayed susceptibility towards *Candida albicans* and *Streptococcus pyogenes* in a manner that suggests a preference towards particular bacteria than fungi. The exhibited antimicrobial spectrum of the crude root extracts is linked to the bouquet of phytochemicals present at varying concentrations. Extensive research revealed that flavonoids possess the inherent ability to deactivate microbial adhesion and cell envelope transport proteins [22, 23]. Other specific

actions of flavonoid rich plants against pathogenic attack include alterations in microbial fluidity as well as the complete distortion of the pathogens' respiratory and intercalation of the microbial nucleotide sequences, thus preventing microbial replication mechanisms [24, 25]. The presence of flavonoids and its synergy with alkaloids alone is highly suggestive of a higher antimicrobial spectrum as alkaloids are reportedly extensively used as pharmaceuticals, psycho-stimulants, narcotics and poisons due to their renowned biologic activities [26]. The activity of alkaloid extracts alone have been investigated in plants like *Callistemon citrinus* and *Vernonia adoensis* whereby at concentrations of 1.7mg/ml, it inhibited the growth of *Staphylococcus aureus* with comparable effects to ampicillin [27]. Other possibilities include the synergies between flavonoids and tannins, both of which have been studied and found to be active against *Staphylococcus aureus* alongside a host of bacterial pathogens linked with respiratory infections [19, 28]. Phytochemicals separated on the basis of solvent extractions (Table 3) revealed that the Ethyl acetate fractions is best suited for treating *S. aureus* (28mm), *K. pneumonia* (30mm), *C. ulcerans* (29mm), *C. krusei* (31mm) and *C. tropicalis* (30mm). As depicted in the results shown (table 2), the roots of *Waltheria indica* Linn is endowed with natural resources to tackle *C. ulcerans* infections, thus indicating a possibility for treating other infections caused by glutamine-rich pathogens like *Mycobacterium tuberculosis*, thus could be used in treating tuberculosis infections. Tables 4 and 5 data shows the MIC, MBC and MFC of the different extracts of *Waltheria indica* Linn. The ethyl acetate fraction of the roots had the lowest consistent MIC value (Table 4) against all clinical strains tested than that of any other solvent fraction (0.25mg/ml). The minimum bactericidal and fungicidal concentrations were the same for *K. pneumonia* and *C. krusei* (0.5mg/ml for all solvent fractions) and within the 0.5-1.0mg/ml range for all tested fractions and microorganisms (Table 5).

5. Conclusion

The present study has shown the phytochemical composition and anti-microbial activity of *Waltheria indica* Linn root. The results authenticates indigenous claims for ethno-medicine in developing countries in addition to improving our depth of knowledge on plant materials useful

in the treatment of upper respiratory tract infections. The discoveries in this study provides scientific support for the antimicrobial spectrum from the phytochemical elements within the root extracts of *Waltheria indica* Linn where the solvent fractions were found to have a significantly high zone of inhibition values when compared to standard drugs ciprofloxacin and fluconazole.

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