In Vitro Antioxidant and Antimicrobial Activities of *Mondia whitei* (Hook. f.) Skeels

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Abstract: *Mondia whitei* (Periplocaceae) is an aromatic plant used as aphrodisiac and for the treatment of urinary infection, jaundice, headache and diarrhoea in Nigeria. The plant was screened for phytochemical components using standard techniques. The antioxidant and antimicrobial activities of the plant were evaluated. The free radical scavenging activity was determined by 2, 2-diphenyl-1-picryl hydrazyl (DPPH) assay. The antimicrobial activity of *M. whitei* extracts against nine clinical isolates was determined by agar well diffusion method. Data were statistically analysed. The root was richer in saponins and tannins than the leaf whereas the leaf contained more flavonoids. The root gave 47.23% inhibition against $DPPH^+$. There was positive correlation between the polyphenolic content and the antioxidant activity of the plant parts. At 300mg/ml, the water extracts of both the leaf and root were more active than the ethanolic extracts. The higher antimicrobial activity of the water extract could be attributed to the solubility of active constituents of the plant in water. The root showed higher antioxidant and antimicrobial activities than the leaf. *M. whitei* root could be very effective in the management of metabolic and infectious diseases. However, toxicological studies will confirm its safety in administration.

Keywords: *Mondia whitei*, Antioxidant capacity, Antimicrobial activity, Polyphenolic content, Nigeria.

INTRODUCTION

Free radicals and other reactive oxygen species are derived from normal essential metabolic processes in the human body or from exogenous sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals [1]. Free radicals play a key role in the aging process and in disease incidence and progression. An antioxidant is a molecule stable enough to donate electron to a rampaging free radical and neutralize it, thus reducing its ability to damage cells. Synthetic antioxidants are used majorly in food industries as preservatives; their use, however, has been restricted lately due to high toxicity levels and carcinogenic effect [2]. Naturally occurring antioxidants have multiple health benefits, are more readily accepted by the body, and their effects are noticed in human tissues. The consumption of dietary antioxidant plants has shown good results in the prevention of human diseases [3]. Some dietary sources of antioxidants are: soybean (isoflavones and phenolic acids); green or black tea (polyphenols and catechins); coffee (phenolic esters); red wine (phenolic acid); citrus and other fruits (bioflavonoids and chalcones); onions (quercetin and kaempferol); olive (polyphenols); Mustard or turmeric (curcumin); and mushrooms (canthaxanthin) [4, 5].

There are reports on anti-radical activity of plant secondary metabolites. A novel flavonoid, 2-(2, 4-dihydroxy-phenyl)-3, 6, 8-trihydroxy-chomen-4-one, isolated from the roots of *Plumbago zeylanica* exhibited antioxidant activity [6]. Polyphenol extracts from *Aframomum melegueta, Zingiber officinalis* and *Myristica fragrans* exhibited antiglycation and antioxidant potentials [7]. Muralidhar *et al*. [8] reported anti-radical activity of bioactive components in *Decalepis hamiltonii*. Also, reactive scavenging activity of the total phenolic content from different parts of *Carica papaya* was reported by Maisarah *et al.* [9].

M. whitei (Periplocaceae) is an aromatic soft woody climber of drier forest with pale greenish-white or cream flowers. The large tuberous root stock is commonly known as “isirigun” by the Yoruba ethnic group of Nigeria. The plant is widely distributed in tropical Africa, from Guinea through Cameroun to East Africa [10, 11]. In Sudano-guinea zone, it grows as a large woody liana with a pleasant vanilla smell [12]. Traditionally, it is a valuable plant in the management of diseases [13, 14]. It is effective in the treatment of malaria, premature ejaculation, and low sperm production, loss of appetite, gonorrhoea, paediatric asthma, and gastrointestinal disorder [15, 16]. In Imo state of Nigeria, the fruits are eaten as a vegetable whereas the whole plant is boiled and used in the treatment of pile [17]. One teaspoonful of the root extract 3 times daily is used for de-worming children [18]. The antimicrobial effect of *M. whitei* root has been reported by previous authors [19-21]. Also, Mayunzu *et al*. [20] reported the antioxidant activity of various concentrations of the root extract.

In view of a dearth of information on the antioxidant and antimicrobial activities of the regenerative parts of

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Mondia whitei, this study compared the *in vitro* antioxidant and antimicrobial activities of the leaf and root of *Mondia whitei*, to provide more scientific insight into its traditional and sustainable use as remedy for the treatment of diseases.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Whole plants of *M. whitei* were collected from a forest in Oyo town along Ibadan-Ogbomoso road, Nigeria. Identification was done at the University of Ibadan Herbarium (UIH) where voucher specimen was deposited (UIH 22405). The samples were powdered and stored at 4°C for further use.

Phytochemical Analyses

The powdered leaf and root were screened for the presence of alkaloids, flavonoids, polyphenols, saponins, and tannins using standard techniques [22-24].

Antioxidant Capacity

The antioxidant activity was examined using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) assay. Ten grams (10g) of powdered sample was extracted in 100ml of methanol to give an extract concentration of 100mg/ml; 0.2ml of extract was added to 2.8ml of freshly prepared 20mg/dm^3 DPPH in methanol, and incubated for 20min in the dark at room temperature. Absorbance was determined using UV/VIS SHIMADZU brand UVmin1240 spectrophotometer at 517nm. Methanol only was used as the blank to adjust the spectrophotometer to zero absorbance whereas DPPH in methanol was used as the control. DPPH is a commercially available stable free radical which is purple in colour. Antioxidant molecules present in samples, when incubated, react with DPPH and convert it into di-phenyl hydrazine which is yellow in colour. The degree of decolourization can be expressed as the % of DPPH radicals scavenged (% inhibition of DPPH$^+$) when the absorbance is measured with a spectrophotometer. The ability of the extracts of the leaf and root to inhibit DPPH radical was calculated [25] as follows:

$$\% \text{ Inhibition} = [1 - (A_{sample} / A_{control})] \times 100$$

$A_{control}$ is Absorbance for control and A_{sample} is Absorbance for test sample.

Preparation of Plant Extracts

Ethanolic Extraction

One hundred grams (100g) of each dried powdered sample (leaf/root) was extracted in 80% ethanol (500ml) for 48h. The extract was filtered (with Whatman No 1 filter paper) and evaporated to dryness at 40°C using a rotary evaporator. The extract was refrigerated at 4°C prior to use.

Water Extraction

One hundred grams (100g) of each dried powdered sample (leaf/root) was soaked in 500 ml of distilled water for 48 h. The mixture was filtered and the filtrate was freeze-dried. Extract was refrigerated at 4°C prior to use.

Organisms

The organisms were clinical isolates of *Bacillus cereus*, *Escherichia coli*, *Candida albicans*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus pyogenes* obtained via due process from University College Hospital (UCH) Ibadan. The isolates were maintained on nutrient agar and grown in nutrient broth for 18hours at 35 ± 2°C for experiments.

Antimicrobial Assay

One gram (1g) and 3g each of the extract was reconstituted in 10ml each sterile distilled water to obtain a concentration of 100mg/ml and 300mg/ml respectively that were used for the antimicrobial assay. The extracts were screened using agar well diffusion assay [26]. 19ml of nutrient agar was inoculated with 1ml of overnight cultures (10^1 - 10^6 cfu/ml) of the test organisms. The inoculated agar was then poured into Petri dishes and allowed to set. From each of the plates, four wells were bored in the agar using 6mm sterile cork-borer; 100µl of extract was introduced into each of the wells using a micro-pipette. The plates were left at room temperature, long enough for diffusion of the extract into agar. Subsequently, the plates were incubated at 35 ± 2°C for 18-36h. Zones of inhibition were recorded in millimetres. Each experiment was carried out three times for all the organisms.

Data Analysis

Data were expressed as mean ± SD. Difference between means was assessed for significance at P≤0.05 by Duncan's Multiple Range Test (DMRT).

Table 1: Quantitative Phytochemical Components of Powdered Leaf and Root of *M. whitei*

Phytochemical (mg/100g)	Leaf	Root
Alkaloids	636.67 ± 10.41	656.67 ± 16.07
Flavonoids	1383.33 ± 10.41	615.00 ± 15.00
Saponins	848.33 ± 12.58	1346.67 ± 16.07
Tannins	740.00 ± 15.00	1221.67 ± 28.43
Polyphenols (mg GAE/g)	416.67 ± 10.41	573.33 ± 12.58

Values represent the mean ± SD; n=3; GAE = gallic acid equivalent.

RESULTS

Phytochemical Components of Samples

M. whitei leaf and root contained alkaloids, flavonoids, polyphenols, saponins, and tannins (Table **1**). The alkaloids (656.67 mg/100g), saponins (1346.67 mg/100g), tannins (1221.67 mg/100g) and polyphenols (573.33 mg GAE/g) were higher in the root than the leaf. The flavonoids (1383.33mg/100g) were significantly higher in the leaf than the root.

Antioxidant Capacity of Extracts of *M. whitei*

The polyphenolic content and antioxidant activity of the two samples is presented in Table **2**. The polyphenols were higher in the root than the leaf. The % inhibition of DPPH free radicals was 32.57% for the leaf and the root had 47.23%. The high antioxidant activity of the root could be attributed to its polyphenolic content.

In Vitro Antimicrobial Activity of *M. whitei* Ethanol Extracts Against Nine Pathogenic Organisms

The extracts (100mg/ml) of the two samples were initially screened for antimicrobial activity against organisms (10^3cfu/ml). The extracts gave very low or no antimicrobial activity. Based on the results of the initial screening the concentration of the extracts was increased to 300mg/ml and the inoculum load was reduced to 10^5- 10^6 cfu/ml for further screening. At 10^5cfu/ml, the ethanol extract (300mg/ml) of the root was active against 7 out of 9 organisms. It was inactive on *Candida albicans* and *Klebsiella pneumoniae*. The highest inhibition was on *Pseudomonas aeruginosa*

Table 2: *In Vitro* Antioxidant Activity of *M. whitei* Against DPPH$^+$

Plant part	Decolourization	Polyphenols (mg GAE/g)	% Inhibition of DPPH$^+$
Leaf	++	416.67±10.41	32.57±0.32
Root	+++	573.33±12.58	47.23±0.35

++ = moderate; +++ = high. Values represent mean ± SD; n=3.

Table 3: Inhibitory Effect of Root Extracts of *M. whitei* Against Pathogenic Organisms

Organism (10^5 cfu/ml)	Plant extract/Zone of inhibition (mm)	
	Root (Ethanol)	Root (Water)
Bacillus cereus	10.33±0.58^a	14.0±0.82^a
Escherichia coli	18.33±1.53^c	16.33±3.20^b
Candida albicans	Na	18.00±2.00^b
Klebsiella pneumoniae	Na	12.67±3.06^a
Pseudomonas aeruginosa	25.00±1.00^e	16.67±1.53^b
Proteus mirabilis	21.00±1.00^d	16.33±1.53^b
Salmonella typhi	15.00±1.05^b	17.67±2.52^b
Staphylococcus aureus	18.67±1.15^c	17.67±0.58^b
Streptococcus pyogenes	24.33±0.58^e	Na

Values are mean ± SD; n=3; values in the same vertical array followed by the same letter are not significantly different ($p<0.05$). Na = Not active; diameter of cork-borer = 6mm.

Table 4: Inhibitory Effect of Leaf Extracts of *M. whitei* Against Pathogenic Organisms

Organism (10^5 cfu/ml)	Plant extract/Zone of inhibition (mm)	
	Leaf (Ethanol)	Leaf (Water)
Escherichia coli	16.67±1.53[a]	Na
Candida albicans	Na	19.33±1.15[a]
Klebsiella pneumonia	18.67±1.15[a]	21.67±0.58[a]
Pseudomonas aeruginosa	Na	21.33±1.15[a]
Salmonella typhi	Na	20.0±2.00[a]
Staphylococcus aureus	Na	20.67±2.31[a]
Streptococcus pyogenes	Na	19.30±1.15[b]

Values are mean ± SD; n=3; values in the same vertical array followed by the same letter are not significantly different ($p<0.05$). Na = Not active; diameter of cork-borer = 6mm.

(25.0mm) and least on *Bacillus cereus* (10.3mm) (Table **3**). The root water extract was also active against all the isolates except *Streptoccocus pyogenes* with highest inhibition on *Escherichia coli* (18.0mm) and least on *Klebsiella pneumoniae* (12.7mm). Overall, the root water extract exhibited antibacterial and antifungal activity.

At 10^6cfu/ml, the ethanol extract of the leaf was active against 2 out of 9 organisms (Table **4**). It was active against *Escherichia coli* (16.67mm) and *Klebsiella pneumoniae* (18.67 mm). The leaf water extract was active against 6 out of 9 organisms. The highest inhibition of 21.7mm was against *Klebsiella pneumoniae* and least (19.3mm) was against *Streptococcus pyogenes.*

DISCUSSION

In this study, the root and leaf of *M. whitei* contained alkaloids, flavonoids, saponins, tannins, and polyphenols in varied quantity. The finding on phytochemical components is in line with an earlier report on the plant. *M. whitei* root extract contained saponins, tannins, alkaloids and phenols; moderate anthranal glycosides, it also contained weak flavonoids and terpenoids whereas the reducing sugars tested negative [10]. The therapeutic values of plant secondary metabolites have been reported by previous authors. The pharmacological actions of alkaloids include: analgesics and narcotics, central nervous system stimulant, mydriatric, miotics and antihypertensive [27]. Some medicinal plants containing flavonoids have thrombotic and vasoprotective potentials; others are used to inhibit tumour formation and/or growth; some have diuretic, antifungal or antibacterial properties, as well as antispasmodic potentials. Flavonoids are also valuable in the maintenance of membrane integrity through their scavenging or chelating abilities [28]. Natural phenolics manifest their activity by decreasing oxygen concentration and intercepting singlet oxygen, hence preventing the formation of free radical [29]. Polyphenols therefore play a key role in the overall antioxidant activity of medicinal plants.

Antioxidant capacity evaluation is important as this may support the claimed uses of many medicinal plants in folk medicines. The relatively high percentage of inhibition of $DPPH^+$ by the root (47.23%) supports its use in traditional herbal medicine. The polyphenols in the root could be responsible for higher free radical scavenging activity. The antioxidant activity of the samples was slightly lower when compared with commercial and synthetic antioxidants such as BHT (49%) and α-tocopherol (58%). Although oxygen radical absorbance capacity (ORAC) method is preferred for the measurement of antioxidant activity of food and biological samples [30], the DPPH assay provides reliable information on the reactivity of test sample with a stable free radical and produces strong absorption band when measured at 517nm. The degree of reduction of free radicals in DPPH is suggestive of the antioxidant power of the sample or extract screened. The present result on the antioxidant activity of *M. whitei* agrees with the report of an earlier study by Mayunzu *et al*. [20].

There is a dearth of information regarding the antimicrobial activity of *M. whitei* in the literature probably because the root part of the plant is majorly used as an aphrodisiac in many African countries, apart from its usefulness in the treatment of anorexia, bilharzias, stress and sexual dysfunction [31]. The findings on the antimicrobial activity of *M. Whitei* are in

line with previous reports on the plant. Fankam *et al*. [19] reported the antibacterial activity of methanol extract of the root against *E. coli*, *K. pneumoniae* and *P. aeruginosa*. Also, Okitoi *et al*. [32] reported antimicrobial activity of water extract from the root against *Escherichia coli* and *Salmonella typhi.* In the present study, only the water extracts gave significant antimicrobial activity whereas the root part was more active than the leaf. This suggests that the phytochemical components of the plant parts are more soluble in water than in ethanol.

CONCLUSION

M. whitei root showed significant antioxidant and antimicrobial activities, the observed activities could be attributed to the presence of phytochemicals such as alkaloids, polyphenols and flavonoids. The antioxidant activity of the root is an indication that the plant could be useful in the management of metabolic diseases such as diabetes, heart diseases and various cancers. Also, the antimicrobial activity confirmed the potential of the root in the treatment of infectious diseases. Overall, the findings of this study justify the use of *M. Whitei* root in traditional medicine in Nigeria.

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