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EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF *MONDIA WHYTEI* ROOTS EXTRACTS

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Extracts of *Mondia whytei* (*M. whytei*) root barks were screened for their inhibitory effects against *Staphylococcus aureus* (*S. aureus*) (ATCC 25923), *Escherichia coli* (*E. coli*) 0157:H7 (PSSCMI 0032), *Bacillus subtilis*, *Candida albicans and Aspergillus niger* were used as test organisms. From the results it was concluded that activity varied with the solvent used. Contrary to previous reports, the plant seems to lack significant antibacterial activity except against *E. coli*. The popularity of a herbal recipe is not always a measure for its potency. However, *M. whytei* had antifungal activity since the ethanol and methanol extracts showed significant activity against the tested strains of fungi. The antioxidant activity of the extracts was also evaluated using the DPPH free radical scavenging assay. *M. whytei* exhibited substantial inhibition of the DPPH activity with EC_{50} of 413mg/l for the crude extracts. This antioxidant activity of the crude extracts can be attributed to the presence of 2-Hydroxy-4-Methoxybenzaldehyde that is a known antioxidant in the root extracts.

Keywords: antimicrobial activity, antioxidant activity, bioactive plant products

Introduction

M. whytei belongs to the milk-weed family; it grows in forests, bush lands and wastelands and is a deciduous canopy-climbing liane (Shitanda, 2007). It is widely used in Eastern, Central and Southern Africa as a flavoring sweetening agent and in herbal medicine (Musonthi, 1991). The roots have been used for the treatment of intestinal disorders, hangovers and detoxification (Njihia, 2005). Pharmacological studies have shown that the root barks have larvicidal activity against Malaria causing parasites (Musonthi, 1991). Other reported bioactivities of *M. whytei* include exhibition of serotogenic properties and 5 – HT receptor agonistic effects (Githingi, 2004). The main flavoring compound identified was 2- hydroxyl – 4 –

methoxy benzaldehyde. This compound also has insecticidal properties (Kubo *et al*., 1999).

Food borne infections have been one of the major public health concerns and account for considerable high cases of illness (Voravuthikunchai *et al*., 2006). The number of invasive fungal and bacterial infections has dramatically increased in both developed and developing countries (Kisangau, *et al.,* 2009). In Africa, traditional medicine is used for the treatment of many diseases and infections. Plants readily synthesize substances for defence against the attack of insects, herbivores and microorganisms (Marjorie, 1999). Recently, there has been an upsurge in demand for natural products for the control and treatment of various infections and diseases as some chemically synthesized drugs have undesirable effects (Hammer *et al.,* 1999).

The intake of antioxidant compounds present in food is an important health protecting factor (Fogliano *et al.,* 1999). The use of antioxidant rich diets leads to a limited incidence of cardio and cerebrovascular diseases and protects the body from free radicals (Hertog *et al*., 1993). Although many antioxidants are obtained from foods sources, such as fresh fruits and vegetables, it is difficult to get enough from these sources (Balch, 2006). Antioxidants used in the food industry are synthetic mainly, ascorbic acid, butylated hydroxy toluene (BHT) and butylated hydroxyl anisole (BHA). However, synthetic antioxidants may be carcinogenic (Tsuda *et al.,* 1994). Natural antioxidants include ά-tocophenol and ß-carotene (Kahkonen *et al*., 1999).

The present study was undertaken to assess the activity of *M. whytei root* extracts against common food borne pathogens and to evaluate the antioxidant properties of the extracts.

Materials and methods

Preparation of the Plant Extracts

Fresh *M. whytei* root barks were harvested from several selected plantations in Kakamega district, Kenya. The roots were washed, peeled and immediately transported to Jomo Kenyatta University, Kenya for analysis. The root barks were dried at temperature of 29°C in a hot air oven for seven days and milled into fine powder. The extracts were prepared by cold maceration technique. The dried root powder was percolated with 100% water, ethanol and methanol separately. The soaked powder was kept at room temperature for 36 hours and filtered using a centrifuge (Shimandzu model). The extracts obtained were concentrated in a vacuum at temperature of 40^0C using a rotary evaporator (Eyala model) and then concentrated to dryness using a freeze drier.

Antibacterial Activity

The antibacterial activity was determined using the hole-in-plate bio assay procedure (Hugo *et al.,* 1983; Vlientick *et al.,* 1995). The pure cultures of the microorganisms were inoculated onto Muller-Hilton nutrient broth incubated at temperature of 37°C for 24 hours. Using a sterile cork-borer of 5mm diameter,

three holes were made into the Petri dishes seeded with bacterial culture. Concentrations of 0.1, 0.2 and 0.3 g/ml extracts were reconstituted in distilled water and transferred into the wells. The plates were incubated at temperature of 37°C for 18 hours. *S. aureus* (ATCC 25923), *E. coli* 0157:H7 (PSSCMI 0032), *Salmonella typhi* (PSSCMI 0034) and *Bacillus subtilis* were used as the test microorganisms. All bacterial cultures were maintained on nutrient agar slants at temperature of 4ºC and sub cultured onto nutrient agar broth for 24 hours prior to testing. The plates were kept for 30 min at room temperature to allow diffusion of the extract, and then were incubated at temperature of 37º C for 18 hours. After the incubation period, the zones of inhibition will be measured using a caliper. Studies were performed in triplicates and the mean value were calculated. The mean zones of inhibitions were compared by one way analysis of variance. The extract concentrations that exhibited the highest activity were diluted double fold (2:2) with nutrient broth agar in a series of twelve test tubes. An aliquot of 1ml of bacterial suspension was inoculated in each tube. The control tube was inoculated with the same quantity of aqueous sodium benzoate. All tubes were incubated at temperatures of 37ºC for 24hours. The lowest concentration that not permits any visible growth when compared to the control was considered as the minimum inhibitory concentration (MIC).

Antifungal Activity

Holes were made into the Petri dishes containing inoculated medium as described by (Vlientick *et al.,* 1995). Extracts concentrations of 0.1, 0.2, 0.3 g/ml were powered into the wells and examined against *Candida albicans* and *Aspergillus niger*. The diameter of the clear zone around the wells (inhibition diameter) was measured at the end of the incubation period. The extracts that presented high mean diameter were subjected to minimum inhibitory concentration (MIC) analysis as described above. Three extracts wells per plate against a single microorganism were used.

Antioxidant activity

M. whytei plant extracts for antioxidant activity analysis were prepared according to the methods described by Aderogba *et al.* (2004). The high antioxidant activity in plants is often associated with the presence of phenolic compounds (Thabrew *et al.,* 1998; Aderogba *et al.,* 2004). The dried root powder was percolated with ten volumes of methanol (100 %) at room temperature for 36 hours and filtered using a centrifuge (Shimandzu model). The extracts were then concentrated in vacuum. The scavenging effect on DPPH radical was measured according to the methods reported by Mensor *et al.*(2001). A quantity of 0.25 mM methanoic DPPH was added in varying concentrations to the *M. whytei* extract (250, 125, 50, 25, 10 and 5 µg /mL). The mixture was then kept in a dark chamber for 30 minutes. The changes in color were then measured at 514 nm on a spectrophotometer (Brookfield model). The decrease in absorbance was then converted to percentage antioxidant activity and expressed as AA % by using the following formula (Aderogba *et al.,* 2004):

$$AA\ \% = 100 - [((Abs\ sample - Abs\ blank) \times 100)] / Abs\ control].$$

The negative control consisted of methanoic DPPH solution, while the positive control was prepared in the same way as the sample using pure ascorbic acid.

Results and discussion

Antimicrobial properties are useful tools in the control of microorganisms especially in the treatment of infections and food spoilage (Abaoba, *et al,* 2004). Aqueous, methanol, ethanol extracts of the root bark powder were screened for their *in vitro* inhibitory effects against certain strains of fungi and bacteria. Many plants contain microbial inhibitors (Voravuthinkuchai, 2006). MIC values obtained for the tested microorganisms are reported in Tables 1 and 2. The water extract does not present significant activity against all organisms except for *Staphylococcus aureus* where the water extract exhibited the highest activity. However, the ethanol extract presented significant activity against *Candida albicans* and *Aspeigillus niger* with MIC values of 58.59 and 14.65 µg/ml respectively. Methanol extract had high MIC values of less than 14.65 and 14.7 µg/ml fo**r** *Aspergillus niger* and *E. coli* respectively. This suggests that methanol is a good solvent for extracting bio active compounds extracted from *M. whytei* against fungi. From these results, it was observed that extracts bioactivity varied with the solvent used. Contrary to previous reports, the plant seems to lack significant antibacterial activity except for methanol extracts against *E. coli* (Table 2). *M. whytei* had significant antifungal activity (table 1) against the tested strains of fungi. However, naturally occurring combinations of these compounds can be synergistic and often result in the plants having greater antimicrobial activity than extracts (Delaquis *et al.,* 2002).

Table 1. Antifungal activity of *M. whytei* root extracts

	MIC µg/ml		
Microorganism	Methanol extract	Ethanol extract	Water Extract
Candida albicans	-	58.59	-
Asperigillus niger	< 14.65	14.65	-

Table 2. Antibacterial activity of *M. whytei* root extracts

	MIC µg/ml		
Microorganism	Methanol extract	Ethanol extract	Water Extract
E. coli	14.7	937.5	7,500
B. subtilis	15,000	1875	-
S. aureus	-	-	1875
S. typhi	-	468.7	-

Antioxidant activity of the crude methanol extracts from the root powder was evaluated using DPPH free radical scavenging assay *M. whytei* exhibited substantial inhibition of the DPPH activity; with 50 % inhibition (EC_{50}) of 413 mg/l. These results were favourably compared to Ginkgo biloba, a standard antioxidant agent, with an EC_{50} of 40.72 mg/l (Aderogbba *et al.,* 2004). The results

are presented in Figure 1. The results suggest that the crude extracts from *M. whytei* possess significant antioxidant activity as demonstrated by the value of EC_{50}. This antioxidant activity of the crude extracts can be attributed to the presence of 2-Hydroxy-4-Methoxybenzaldehyde that is a known antioxidant in the root extracts (Njihia *et al.,* 2005). Further study *of M. whytei* fractions is necessary in order to isolate, characterize and evaluate the antioxidant and antimicrobial compounds. The findings of this work also clearly demonstrate simple *in vitro* systems employed as reliable systems for the preliminary screening of plants.

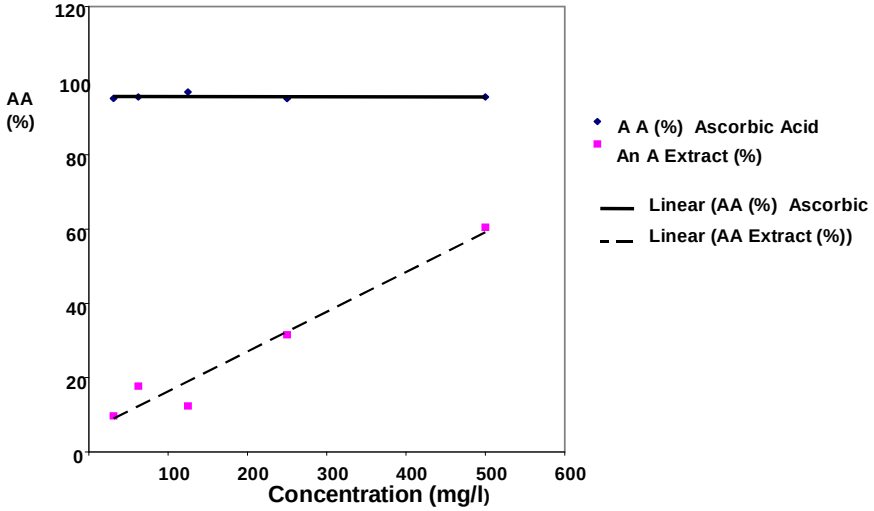

Figure 1. Antioxidant activity of crude *M. whytei* extracts and for ascorbic acid. AA – Antioxidant activity

Conclusions

The results revealed that *Mondia whytei* has the potential to be used as a nutraceutical. The roots presented high antioxidant, antimicrobial effect. Therefore, the use of *Mondia whytei* in product development does not only improve flavor but also increases the nutritional content of the product. This insight could be valuable for products fortified with the root extract in order to have longer shelf-life compared to similar brands.

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