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Research Article

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF ETHANOL AND N-HEXANE EXTRACTS OF *WALTHERIA INDICA* AND *MUCUNA PRURIENS*

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ABSTRACT
The antioxidant and antimicrobial activities of many Nigerian Medicinal plants used in folk medicine have been reported. In the present study extracts of shoots of *Waltheria indica* and *Mucuna pruriens* were evaluated for their antioxidant and antimicrobial activities using 2,2 diphenyl-1-picrylhydrazyl free radical scavenging activity and paper disc diffusion method respectively. The results showed that the n-hexane extracts of Mucuna pruriens and *Waltheria indica* showed the best 2,2 diphenyl-1-picrlylhydroxyl free radical scavenging activities whereas ethanol extracts of *Mucuna pruriens* and *Waltheria indica* gave the best antimicrobial activities, against *Salmonella typhi*, *Bacillus subtillis*, *Streptococci, Escherichia coli, Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* indicating that the plants could be potential sources for antioxidant and antimicrobial compounds needed for the treatment of pathological conditions and various microbial diseases caused by the test organisms.
Keywords: Antimicrobial activity, Antioxidant activity, *Waltheria indica* and *Mucuna pruriens*.

INTRODUCTION

Medicinal plants and herbs are shrubs known to modern and ancient civilizations for their healing properties. They are the sole source of active principles capable of curing man's ailments. Modern pharmaceuticals rely heavily on the same active principles, be they natural or synthetic. With thousands of active principles yet to be discovered or fully evaluated, it is no wonder that biodiversity is a fundamental topic on any nature preservation agenda. Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth (antimicrobial activity) [1, 2]. The substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs. Medicinal plants and herbs continue to be the source of proven medications of new and revolutionary drugs. The active principles of synthetic drugs are also important and can be found in many plants and herbs which are cheaply and readily available.

Chemical compounds and reactions capable of generating potential toxic oxygen species can be referred to as pro-oxidant. On the other hand, compounds and reactions disposing of these species, scavenging them, suppressing their formation or opposing their actions are antioxidants[3]. Researchers have focused on plant natural antioxidants found in phytochemicals such as phenolic compounds, diterpenes, flavonoids and tannins[4,5,6]. These phytochemicals act by removing free radicals in the polar and lipid phase and inhibit different types of oxidizing enzymes[7].

As a result of indiscriminate use of antimicrobial drugs in the treatment of infectious diseases, microorganisms have developed resistance to many antibiotics[8]. Therefore, there is need to develop alternative antimicrobial drugs and one approach is to screen local medicinal plants which represent a rich source of novel antimicrobial agents. The present study was carried out to investigate the antimicrobial and antioxidant properties of two medicinal plants found in Afaka, Nigerian Defence Academy, Kaduna, Nigeria. This screening is of significant importance because of the urgent need for compounds that would be added to or replace the current antimicrobial agents to which microbes have become largely resistant.

MATERIAL AND METHODS

Materials

Materials used in this study include, Ethanol (BDH), n-hexane (BDH), Ascobic acid, α-tocopherol and DPPH.

Methods

Plant collection and identification

The shoots of Waltheria *indica* (Voucher number 900103) and *Mucuna pruriens* (Voucher number 900231) were collected from Kangimi village of Igabi local government Area, Kaduna state during the month of April. The samples were identified and Authenticated by Mallam Yahaya of the herbarium unit of Biological Sciences Department, Nigeria Defence Academy, Kaduna. Voucher specimens were deposited in the herbarium for future reference.

Preparation of the extracts

The stem and leaves of *Waltheria indica* and *Mucuna pruriens* were separately air dried and pulverized using wood mill machine. The pulverized sample were weighed and preserved by rapping in aluminum foil before the extraction. A portion (100g) of each pulverized sample of the dried (stems and leaves) of *Waltheria indica* was extracted separately by percolation in ethanol for seven days and filtered. A portion 150 cm^3 of the filtrate were measured into 500 cm^3 separatory funnel and 150cm^3 of n-hexane (1:1) was added and the mixture was shaken and left over night for the two layers to separate. Both layers were drain and separately evaporated using rotary evaporator at 40^oC. The crude ethanolic and hexane extracts were kept at ambient temperature until needed.

Determination of antioxidant activity

The antioxidant activity or the capacity to scavenge the "stable" free radical DPPH was determined using the DPPH free-radical scavenging activity[9,10]. A portion (3.0mg) of 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH which is a stable radical), was dissolved in 100cm^3 of ethanol, the solution was allowed to stand for 10 minutes and absorbance was measured at 517nm. The solution of the extract was prepared by dissolving 0.49 mg, 0.98 mg and 1.96 mg in 2cm^3 of

ethanol to give an approximate concentration of 0.25, 0.5 and 1.0 mg/cm^3 respectively. A portion 3cm^3 of DPPH solution was added to 0.5cm^{3}of each sample solution, shaken and allowed to stand for 10minutes, after which the decrease in absorption at 517nm was measured.

The decrease in absorption of the test sample was calculated by subtracting that of the control. The same procedure was carried out using vitamin C (ascorbic acid) and α-tocopherol which were used as standard. All test and analysis were carried out in triplicate and the results obtained were averaged. Radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH discoloration using the equation below:

% RSA or % inhibition = { ($A_{DPPH} - A_S$) / A_{DPPH} } x 100.

Where A_s = absorbance of the solution when the sample extract has been added at a particular level to the DPPH

Antimicrobial Assay

The antibacterial activities of crude extracts were determined by using the methods described by [11,12]. A portion (20 mg) of each extract was dissolved in 5 cm^3 of the respective solvents of extraction to give 4000µg/ cm^3 of the stock solution. Using a micropipette, 0.35 cm^3, 0.3 cm^3, 0.25 cm^3 and 0.2 cm^3 of the solution were separately drawn into vials and the volumes adjusted to 2 cm^3 to give approximate concentration of 7x 10^2, 6x10^2, 5x10^2 and 4x10^2 µg/ cm^3 respectively. Filter paper was carefully labeled and cut into sizes of 0.5cm diameter and separately introduced in each vials containing the prepared solution. They were dried at 50^{o}C. A control was similarly set up using distilled water and ethanol and n-hexane that was used in the extraction. Nutrient agar was used as the growth medium for the microbes. Each medium was prepared by dissolving 38g of the agar in 1000 cm^3 of distilled water, heated to dissolve and autoclaved at 120^o C for 15mins. It was then cooled and poured into petri dishes to solidify. Isolates of *Salmonella typhi*, *Bacillus subtillis, Streptococci, Escherichia coli, Aspergillus niger, Aspergillus flavus and Candida albicans* were separately cultured on each nutrient agar plate; sterile paper disc incorporated with the extract were placed on each agar and incubated for 24hrs. The zones of inhibition diameter were measured with the aid of a plastic ruler and the minimum inhibitory concentrations (MIC) determined.

TABLE 1: PERCENTAGE INHIBITION OF DPPH SCAVENGING ACTIVITY OF STEM EXTRACTS OF *WALTHERIA INDICA*

	percentage			
Concentration (mg/cm^3)	A_1	A_2	Ascorbic acid	α – tocopherol
0.25	14.6	21.2	67.3	12.1
0.5	15.0	31.1	68.7	12.2
1.0	26.3	54.5	90.9	15.4

Key: A_1= ethanol extract, A_2= n-Hexane

TABLE 2: PERCENTAGE INHIBITION OF DPPH SCAVENGING ACTIVITY OF LEAVES EXTRACTS OF *WALTHERIA INDICA*

	Percentage inhibitions			
Concentration (mg/cm^3)	A_3	A_4	Ascorbic acid	α – tocopherol
0.25	14.7	24.6	67.8	12.1
0.5	20.7	72.0	68.7	12.2
1.0	38.1	92.8	90.2	15.4

TABLE 3: PERCENTAGE INHIBITION OF DPPH SCAVENGING ACTIVITY OF STEM EXTRACTS OF *MUCUNA PRUREINS*

	Percentage inhibition			
Concentration (mg/cm^3)	A_5	A_6	Ascorbic acid	α – tocopherol
1.0	49.9	33.0	67.3	12.1
0.5	33.2	30.4	68.7	12.2
0.25	32.5	29.9	90.9	15.4

Key: A_5= ethanol extracts, A_6= hexane extract

TABLE 4: PERCENTAGE INHIBITION OF DPPH SCAVENGING ACTIVITY OF LEAVES EXTRACTS OF *MUCUNA PRUREINS*

	Percentage inhibition			
Concentration (mg/cm^3)	A_7	A_8	Ascorbic acid	α – tocopherol
1.0	49.6	79.8	67.3	12.1
0.5	39.4	59.3	68.7	12.2
0.25	29.0	38.3	90.9	15.4

Key: A_7= ethanol extracts, A_8= hexane extract

TABLE 5: RESULTS OF ANTIMICROBIAL ACTIVITY OF *WALTHERIA INDICA*

Zone of inhibition diameter (mm)									
Plants Extract	Extracting Solvents	Conc (µg/l) ($x10^2$)	*Sty*	*Bs*	*Str*	*Ec*	*Af*	*An*	*Ca*
W. indica Stem	n-hexane	4	8	6	5	7	NI	NI	7
		5	12	10	9	9	NI	8	9
		6	16	12	10	13	NI	10	12
		7	20	15	14	16	NI	16	15
		C	NI	NI	NI	NI	NI	NI	NI
Leaves	Ethanol	4	9	10	10	7	NI	NI	NI
		5	13	15	13	13	7	Ni	NI
		6	17	20	18	16	13	7	NI
		7	22	25	26	25	20	9	NI
		C	NI	NI	NI	NI	NI	NI	NI
	n-hexane	4	10	NI	13		NI	7	12
		5	12	NI	16	11	NI	12	14
		6	15	10	21	13	NI	14	16
		7	18	15	23	16	10	16	21
		C	NI	NI	NI	20	NI	NI	NI
	Ethanol	4	7	9	NI	NI	NI	10	NI
		5	12	12	9		NI	12	NI
		6	15	15	13	NI	NI	14	NI
		7	17	21	15	NI	NI	18	NI
		C	N	NI	NI	10	NI	NI	NI
						13			
						NI			

Key: NI = No inhibition, *Sty= Salmonella typhi Bs= Bacillus subtilis, Str= Streptococci, Ec= Escherichia coli, , Af=Aspergillus flavus, An= Aspergillus niger* and *Ca= Candida albicans*

TABLE 6: RESULTS OF ANTIMICROBIAL ACTIVITY OF *MUCUNA PRURIENS*

Zone of inhibition diameter (mm)									
Plants Extract	Extracting Solvents	Conc (µg/l) ($x10^2$)	*Sty*	*Bs*	*Str*	*Ec*	*Af*	*An*	*Ca*
Stem *Mucuna prureins*	n-hexane	4	8	NI	10	10	NI	NI	NI
		5	10	NI	13	12	NI	10	NI
		6	13	NI	15	13	NI	13	12
		7	17	10	17	14	NI	14	15
		C	NI	NI	NI	NI	NI	NI	NI
Leaves	Ethanol	4	NI	NI	11	NI	NI	12	10
		5	10	10	14	10	7	13	14
		6	13	15	16	13	13	15	16
		7	16	20	21	21	20	18	18
		C	NI	NI	NI	NI	NI	NI	NI
	n-hexane	4	NI	NI	10	07	NI	7	12
		5	12	NI	12	10	NI	12	14
		6	15	10	13	12	NI	14	16
		7	16	15	14	15	10	16	21
		C	NI	NI	NI	NI	NI	NI	NI
	Ethanol	4	NI	NI	10	NI	10	10	12
		5	10	10	13	NI	14	12	15
		6	12	15	16	10	18	14	21
		7	16	21	21	14	22	18	25
		C	NI	NI	NI	NI	NI	NI	NI

Key: NI = No inhibition, *Sty= Salmonella typhi Bs= Bacillus subtilis, Str= Streptococci, Ec= Escherichia coli, , Af=Aspergillus flavus, An= Aspergillus niger* and *Ca= Candida albicans*

RESULTS AND DISCUSSION

The results of antioxidant activities of the ethanol and n-hexane extract of stems and leaves of *Waltheria indica* and *Mucuna pruriens* are presented in Tables 1-4. Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases[13]. The extracts in this research exhibited different levels of antioxidant activity. *Waltheria indica* n-hexane leaves extract showed a higher potency than Ascorbic acid and α-tocopherol in scavenging of DPPH free radical. The scavenging activity of n-hexane leaves extracts are generally about six times greater than the one recorded for the synthetic α-tocopherol at the respective concentrations (see Table 2). This is closely followed by the n- hexane leaves extracts of *Mucuna prureins* with DPPH scavenging activity of 79.8% although lower than the standard ascorbic acid but five times greater than synthetic α-tocopherol. Both ethanol and n-hexane extracts of

the leaves showed moderate to low antioxidant activity indicating that the leaves of the two plants possess more potent antioxidant compounds (Tables 1 and 3).

The results of antimicrobial activities showed that all the extracts exhibited high to moderate activities with the ethanol extracts of the stem of *Waltheria indica* exhibiting high activity against *Streptococci*, *Bacillus subtilis* and *Salmonella typhi* with zones of inhibition of 26, 25 and 22mm respectively at a concentration of $7x10^2$ µg/cm^3. Although the ethanol extract was found to be inactive against *Candida albicans* but was moderately active against *Aspergillus flavus*. The n-hexane leaves extracts was only active against *E coli*, *Sterepomycin* and *B. subtilis* with zone of inhibition of 20, 23 and 21 mm respectively but generally the ethanol extract of the of *Waltheria indica* is more active then the leaves (Table 5).

The results of antimicrobial activities of *Mucuna pruriens* showed that the ethanol extracts of both the leaves and stems of the plant are more active when compare with the n-hexane extracts. For instance, the ethanol leaves extract was found to be highly active against *Candida albicans* and *A. flavus* with the zone of inhibition diameter of 25 and 22 mm at a concentration of $7x10^2$ µg /cm^3 respectively. Similarly the ethanol extracts of the stem was active against *Bacillus subtilis*, *Streptococci* and *Escherichia coli* with zones of inhibition diameter of 20. 21 and 21 mm respectively at a concentration of $7x10^2$ µg /cm^3. The n-hexane extracts of both the stem and leaves of *Mucuna pruriens* showed moderate to low activities for all the tested microbes (Table 6).

In conclusion the present study showed that the n-hexane extracts of both *Waltheria indica* and *Mucuna pruriens* could be a source for antioxidant compounds that could be used for the treatment of pathological conditions that are associated the diseases caused by oxidative stress whereas the ethanol extracts could be source of antimicrobial compounds that could be used for the treatment of various diseases caused by the tested microbes.

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