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COMPARATIVE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF FOUR MEDICINAL PLANTS

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ABSTRACT

Pathogenic microorganisms and oxidative stress have continuously threatened the wellbeing of humans. In this study, we determined the Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC)/ Minimum Fungicidal Concentration (MFC) of extracts of *Eugenia uniflora*, *Cassia sieberiana*, *Laportea aestuans* and *Dysoxylum lenticellare* against methicillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis* and *Candida albicans.* The antioxidant activities of these extracts were also evaluated. The 50% methanol extracts were obtained by maceration at room temperature (26-33 °C). The antimicrobial test was carried out by broth dilution assay using Streptomycin and Ketoconazole as positive controls while 50% methanol was used as the negative control. The antioxidant activities were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP) and Total Antioxidant Capacity (TAC) assays with ascorbic acid used as positive control. In anti-bacterial studies, *E. uniflora* was active against all test organisms. *C. sieberiana* had the lowest MIC against *B. subtilis* and MRSA. *Dysoxylum lenticellare* was only active against *B. subtilis*. In antifungal studies, *E. uniflora* and *C. sieberiana* were the only extracts active against *C. albicans*. In antioxidant studies, *E. uinflora* was the most active for DPPH and FRAP assays while *C. sieberiana* was the most active for TAC. In all antioxidant evaluations, *E. uniflora* was the most active followed by *C. siebariana* while *L. aestuans* was the least active. *Eugenia uniflora* had the best antimicrobial and antioxidant activities justifying its ethnomedicinal use in the treatment of microbial infections and free radical- induced conditions such as influenza, digestive disorders, and inflammations.

Key words: Antimicrobial, Antioxidants, Medicinal plants, Minimum inhibitory concentration.

INTRODUCTION

Common course for treatment of infectious diseases often include reducing the burden posed by oxidative stress as well as treatment against causative agents. However, synthesized antioxidants such as Butylated hydroxytoluene (BHT) pose a challenge of toxicity while infection rate and mortality associated with infectious disease is high due to the ever increasing resistance of the pathogens to available antibiotics. This has necessitated the search for potent antioxidant and antimicrobial agents from natural sources especially medicinal plants which are claimed by some traditional healers to be safe and more effective than some existing synthetic antibiotics (Rojas *et al*., 2006)

Laportea aestuans (nettle plant) is employed as remedy against diarrhoea and dysentery (Oloyede and Ayanbadejo, 2014), malaria (Akinniyi *et al*., 1986), liver ailments and toothache (Gill, 1992). Ferulic acid, p–coumaric acid, vanilic acid, and flavonoids: kaemferol, (-)-epigallocatechin, quercitrin and ellagic acid were identified in the leaf extract by GC – MS analysis of *L. aestuans* (Okereke *et al*., 2014). *Cassia sieberiana* (West African laburnum) is employed for various phytotherapeutic purposes. Its root is used in the treatment of hernia, leprosy and ulcer while aqueous extract of the root bark is found to possess antioxidant properties. The root bark extract also inhibited ethanol triggered severe gastric ulcer in rats (Nartey *et al*., 2012). *Eugenia uniflora* (Pitanga) has been applied in folk medicine as an antioxidant, hypotensive, anti-inflammatory and hypoglycemic agent. The leaves

are employed as febrifuges and in the treatment of bronchitis, influenza and intestinal troubles (Consolini and Sarubbio, 2002). Ellagic acid, protocatechuic acid, chlorogenic acid, gallic acid, rutin, vanillic acid, salicylic acid, catechol, catechin, P- hydroxy-benzoic acid, caffeic acid, 3, 4, 5-methoxy-cinnamic acid, ferulic acid, isoferulic acid, alpha coumaric acid, benzoic acid, p-coumaric acid and cinnamic acid were the compounds identified in the leaf extract of *E. uniflora* (Schumacher *et al*., 2015; Bakr *et al*., 2017). Extract of *D. lenticellare* exhibited inhibitory chronotropic effect on rat atrial muscle (Aladesanmi and Ilesanmi, 1987) as well as molluscidal activities (Adewunmi and Aladesanmi, 1988). Phyllocladene, β-hydroxysandaracopimarene, *3-epi*-18-methoxyschelhammericine, *3-epi*-schelhammericine, 2,7-dihydrohomoerysotrine, dysazecine, dysoxyline, homolaudanosine, 3-epi-12-hydroxyschelhammericine and *p*-hydroxyacetophenone were the compounds isolated from the plant (Aladesanmi *et al*., 1983; Aladesanmi,1988).

We report the comparative antioxidant and antimicrobial activities of these four medicinal plants against reference strains of *Escherichia coli* ATCC 25923, *Pseudomonas aeruginosa* ATCC 10145, *Bacillus subtilis* NCTC 8236, methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 29213 and *Candida albicans* ATCC 24433 which are the causative agents of most common infections.

MATERIALS AND METHODS

Plant Materials

The leaves of *Eugenia uniflora* Linn. and aerial part of *Laportea aestuans* (L.) Chew**.** were collected within Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria and was authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan with voucher numbers FHI 102196 and FHI 110350 respectively. The root bark of *Cassia sieberiana* D. C. was collected within Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria and was authenticated at the Herbarium, Department of Pharmacognosy, Faculty of Pharmacy, Faculty of Pharmacy, Ife (FPI), included in the online edition of *Index Herbarorium*) Obafemi Awolowo University, Ile-Ife, Nigeria with voucher number FPI 2158. The stem of *Dysoxylum lenticellare* Gillespie was collected from tropical rain forest of Fiji island (a group of many small islands in the Pacific Ocean between latitude 16°S and 175°E). The plant was collected by George, U. in November 1967 under the collection number 358.

Extraction

The plant materials were dried under ambient condition and were pulverised into powder. Extraction of the plant material was achieved by macerating 100 g of each plant sample with 500 ml of 50% methanol for 72 h. The extracts were filtered with Whatman filter paper No. 1 (Whatman, UK). The filtrates were concentrated to dryness at 40 °C with a rotary evaporator to obtain dry extracts.

Antimicrobial Testing

Source of Microbial Strains

The bacterial and fungal strains used for the antimicrobial screening were obtained from culture collections in the Microbiology Laboratory of the Department of Pharmaceutics, Obafemi Awolowo University. The reference strains used include *Escherichia coli* ATCC 25923, *Pseudomonas aeruginosa* ATCC 10145, *Bacillus subtilis* NCTC 8236, methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 29213, *Candida albicans* ATCC 24433. Bacterial strains were maintained in nutrient broth while the fungal strain was maintained in sabouraud dextrose broth at 4 °C.

Determination of Minimum Inhibitory and Bactericidal/Fungicidal Concentrations

The minimum inhibitory concentration (MIC), the lowest concentration of the test extract to inhibit the growth of the test microorganism; minimum bactericidal concentration (MBC), the lowest concentration of the extract to kill bacterial strain and minimal fungicidal concentration (MFC), the lowest concentration of the extract to kill the fungal strain was determined using micro-broth dilution assay as described by Mahboubi and Haghi (2008) with slight modifications. The nutrient broth was made into varying

concentrations 40.00, 20.00, 10.00, and 5.00, 2.50, and 1.25 mg/ml of the extract and each prepared concentration was inoculated with culture suspensions of 1 x 10^6 CFU/ml (equivalent of 0.50 Mc Farland standard). The bacterial cultures were incubated at 37 °C for 24 h while the fungal culture was incubated at 25 °C for 72 h. The negative control was 50% aqueous methanol while the positive control for bacterial and fungal tests was Streptomycin and Ketoconazole, respectively.

Antioxidant Assays

DPPH Radical Scavenging Assay

Free radical scavenging activity of the extracts was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH) assay method (Aladesanmi *et al*., 2007). Various concentrations (100, 50, 25, 12.5, 6.25 and 3.125 µg/ml) of the extracts were prepared. A 150 µl of 0.5 mM of DPPH solution in methanol was added to 150 µl of each concentration of the extracts in a well plate. Ascorbic acid was used as positive control while methanol was used as negative control. The plate was incubated for 30 min after which the absorbance was measured at 510 nm with UV spectrophotometer (CampSpec M 107 Spectrophotometer, United Kingdom). All tests were carried out in triplicates and the results obtained were expressed as means. The percentage DPPH inhibition was calculated using the equation:

$$\%\text{Inhibition} = \frac{A_{control} - A_{sample} \times 100}{A_{control}}$$

Where $A_{control}$ = Absorbance of negative control (methanol)

A_{sample} = Absorbance of positive control/extracts

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP reagent was prepared following the method of Olawoye and Gbadamosi (2017). A 50 µl aliquot of standard solution of ascorbic acid and test sample at concentrations 20, 40, 60, 80, 100 µg/ml was added and mixed with 1 ml of FRAP reagent in triplicate. Absorbance was taken at 593 nm against blank (distilled water) after 10 minutes of incubation. All tests were performed in triplicate and at room temperature. Data were expressed as mean ± standard error of mean (SEM).

Total Antioxidant Capacity (TAC) Assay

The method of Prieto *et al*. (1999) was used for this study. A 0.3 ml extract was combined with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm at room temperature. Methanol (0.3 ml) was used as the blank. The calibration curve was prepared by mixing ascorbic acid (100, 80, 60, 40, and 20 µg/ml) with methanol. The total antioxidant capacity was expressed as the number of gram equivalent of ascorbic acid. Data were expressed as mean ± standard error of mean (SEM).

RESULTS

Antimicrobial Assay

In this study, we report the antimicrobial activities of *E. uniflora*, *C. sieberiana, L. aestuans* and *D. lenticellare* against reference strains of Methicillin Resistant *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis* and *Candida albicans*. The result of the antimicrobial activities of the extracts is presented in table 1. The inactiveness of the negative control (50% methanol) suggested that activities exhibited by the extracts were not due to solvent used for dissolution. *Eugenia uniflora* extract exhibited inhibitory activities against all tested bacterial strains, but showed the strongest inhibition against *P. aeroginosa* with MIC and MBC of 5 and 10 mg/ml, respectively. Likewise, *C. albicans* extract was moderately susceptible to *E. uniflora* with MIC and MFC of 20 and 10 mg/ml, respectively. Extract of *C. sieberiana* demonstrated inhibitory activity against *B. subtilis* and MRSA, with *B. subtilis* being the most susceptible with MIC and MBC of 10 and 20 mg/ml, respectively. Moreover, *C. sieberiana* extract also showed antifungal activity against *C. albicans*. It was observed that *D. lenticellare* extract was only active against *B. subtilis* while *L. aestuans* extract showed no inhibitory activity against any of the tested bacteria strains. Of all the bacterial strains, *B. subtilis* is the most susceptible especially to *C. sieberiana*. Interestingly, *C. albicans* and MRSA were only susceptible to *E.*

uniflora and *C. sieberiana* extract. In summary, the most active extract was *E. uniflora* followed by *C. sieberiana* while *L. aestuans* extract was the least active against on all tested pathogens. The MBC values were always twice the MIC value except for *E. uniflora* and *D. lenticellare* extracts against *B. subtilis* as well as *C. sieberiana* extract against *C. albicans* where the MIC is equivalent to MBC/MFC. In contrast, *E. uniflora* extract had MIC value which was twice the MFC value against *C. albicans*. In the control test carried out with standard antibiotics, it was observed that Streptomycin and Ketoconazole showed better activity against tested bacterial and fungal strains respectively than all the samples tested.

Table 1: Antimicrobial Activities of selected Medicinal plants

	Test organisms and control/ Concentration (mg/ml)									
	E. coli		***P. aeruginosa***		***B. subtilis***		***S. aureus***		***C. albicans***	
Plant Extract	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MFC**
Eugenia uniflora	10	20	5	10	20	20	20	10	20	10
Laportea aestuans	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
Cassia sieberiana	>40	>40	>40	>40	10	20	10	20	20	>20
Dysoxylum lenticellare	>40	>40	>40	>40	20	>20	>40	>40	>40	>40
Streptomycin	MIC = 0.256 mg/ml								ND	ND
Ketoconazole	ND	ND	ND	ND	ND	ND	ND	ND	MIC=0.1mg/ml	
Negative control	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40

ND: Not determined.

DPPH Radical Scavenging Assay

Our present study showed that the DPPH scavenging capacity of the extracts is concentration-dependent as presented in table 2. Ascorbic acid showed the highest DPPH radical scavenging activity of all the tested samples. Among the plant extracts, *E. uniflora* extract had the highest activity with IC_{50} of 27.47 μg/ml while *L. aestuans* extract had the least activity. Moreover, *C. sieberiana* and *D. lenticellare* extracts were next in activity to *E. uniflora* extract with IC_{50} of 33.15 and 74.94 μg/ml respectively.

Table 2: Antioxidant Activities of selected Medicinal plants

Extracts	**DPPH**	**FRAP**	**TAC**
	IC_{50} ± SEM (μg/ml)	**Mean C ± SEM (AAE μg/g Extract)**	**Mean C ± SEM (AAE μg/g Extract)**
Eugenia uniflora	27.474 ± 1.761	109.302 ± 0.761	162.653 ± 2.863
Laportea aestuans	376.433 ± 2.335	27.006 ± 2.086	64.263 ± 1.908
Cassia sieberiana	33.151 ± 0.581	57.534 ± 0.282	186.43 ± 3.678
Dysoxylum lenticellare	74.939 ± 1.890	101.821 ± 0.524	81.603 ± .657
Ascorbic acid	13.095 ± 0.062	-	-

AAE = Ascorbic Acid Equivalent

Ferric Reducing Antioxidant Power (FRAP) Assay

We expressed activities of the plant in ascorbic acid equivalent (AAE) as presented in table 2. In this study, only two extracts exhibited high antioxidant power. Extract of *E. uniflora* had the highest antioxidant power with AAE of 109.302 μg/g followed by *D. lenticellare* with AAE of 101.821 μg/g. However, extract of *L. estuans* had the least activity of all the extracts tested.

Total Antioxidant Capacity Assay

The result of the total antioxidant capacity of the extracts is summarised in table 2. The antioxidant activities of the extracts are also expressed in ascorbic acid equivalent. Extract of *C. sieberiana*

had the highest total antioxidant capacity with AAE of 186 µg/g followed by *E. uniflora* extract with AAE of 162.653 µg/g. We observed that *L. aestuans* extract was again the least active among the tested medicinal plants. The total antioxidant capacity of *C. sieberiana extract* was three times more than the capacity of *L. aestuans extract.*

Table 3: Ranking of Plant Extracts by their Antioxidant activities

	Ranking			
	DPPH (2,2-diphenyl-1-picrylhydrazyl)	**FRAP** (Ferric Reducing Antioxidant Power)	**TAC** (Total Antioxidant Capacity)	**Overall**
Eugenia uniflora	1	1	2	1
Laportea aestuans	4	4	4	4
Cassia sieberiana	2	3	1	2
Dysoxylum lenticellare	3	2	3	3

DISCUSSION

Various scientific evidences suggest that free radicals play important role in aetiology of infectious diseases and other diseases of ethnomedicinal importance (Pham-Huy *et al*., 2008). However, antioxidants are capable of scavenging free radicals or stabilize free radical by donating hydrogen atom (Brewer, 2011). Plants could therefore afford therapeutic compounds having antioxidant and antimicrobial compounds (Ullah and Khan, 2016) which necessitated our present study. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a simple assay widely used to evaluate antioxidant activities of test samples as well as in quantifying antioxidants in various samples (Olawoye and Gbadamosi, 2017) and it is believed to correlate with other antioxidant assays including TAC and FRAP which is often used to measure the antioxidant capacity of medicinal plants, foods, beverages and nutritional supplements containing polyphenols (Pisoschi and Negulescu, 2011).

In this study, it was observed that extract of *E. uniflora* had the highest antioxidant activity in both DPPH and FRAP assays and second highest in TAC assay and was therefore ranked as the most active plant extract as presented in table 3. The antioxidant activities exhibited by *E. uniflora* could be due to the presence of phenolics and flavonoids which are known to be efficient free radical scavengers (Kadiri and Olawoye, 2016). Quercetin, a known antioxidant compound was reported to be present in the extract of *C. sieberiana* as well as 3-O-rhamnosides of myricetin (Asase *et al*., 2008). These compounds could be responsible for the antioxidant activities displayed by its extract in this study. There were reports on the antioxidant potentials of *L. aestuans* (Oloyede, 2011; Okereke and Elekwa, 2014; Oloyede and Ayanbadejo, 2014) however, we observed that its activities were lower when compared to other extracts and positive control used in this study. The choice of solvents and differences in extraction methods were known to influence antioxidant activity of plant extracts (Ertürk *et al*., 2016; Olawoye and Gbadamosi, 2017), this could be a possible explanation for the differences observed with *L. aestuans.*

Previous antimicrobial evaluation showed that extract of *E. uniflora* showed broad spectrum of antimicrobial activity against gram positive and gram negative bacteria species (Adebajo *et al*., 1989; Fiuza *et al*., 2008; Oliveira *et al*., 2008; Silva-Rocha *et al*., 2015) which is in agreement with the result of this present study. Our findings further corroborate the traditional use of the leaves for the treatment of infections. Inhibitory and/or bacteriocidal/fungicidal activities of the plant extracts could be attributed to phenolics which are known to cause cell death through distortion of cell permeability and coagulation of cell content (Borges *et al*., 2016). The observed inhibitory activity of *E. uniflora* against MRSA suggests it

could be useful source of alternatives in curbing the prevalence of antibiotic resistance of pathogens.

Extract of *C. sieberiana* also exhibited inhibitory activity against *B. subtilis*, MRSA and *C. albicans*. Its observed activity against *B. subtilis* was in contrast to the findings of Asase *et al*. (2008). The differences could be attributed to the use of non-identical strains of *B. subtilis*, however, its inactivity *against Pseudomonas syringae* as reported by Asase *et al*. (2008) agrees with this present study where *C. sieberiana* extract is inactive against *P. aeroginosa*, a closely related species. Previous reports showed that extract of *L. aestuans* exhibited some level of antimicrobial activities (Adebajo *et al*., 1991; Oloyede and Ayanbadejo, 2014) however, it exhibited lower antimicrobial activities when compared to the antimicrobial activities elicited by other extracts and standard drugs evaluated in this study. Various factors including solvent of extraction and microbial strains used could be responsible for disparity in the reports (Ullah and Khan, 2016).

CONCLUSION

Considering all the activities evaluated in this study, *E. uniflora* extract is the most active and could be considered as potential source of compounds with antioxidant and antimicrobial activities. Future studies should be carried out to isolate and identify compounds responsible as well as demonstrate their safety and effectiveness in clinical trials.

Conflict of interest

We declare that we have no conflict of interest.

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