Arch. Bas. App. Med. 10 (2022):123– 128
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Research Article

Evaluation of Antioxidant and Antimicrobial Activities of *Euphorbia graminea* L. (Euphorbiaceae) and *Tetracera scandens* L. (Dilleniaceae)

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Accepted: September 17, 2022

Abstract

Medicinal plants have been used for the treatment of pathogenic infections and protection against oxidative stress. These plants produce a wide range of bioactive chemicals, including defensive properties, that are required for their survival in their natural habitat. The antioxidant and antibacterial properties of *Euphorbia graminea* L. and *Tetracera scandens* L. were investigated in this study. The ability of methanol extracts of both plants to scavenge free radicals was determined using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) chemical assay, total phenolic contents (TPC) was determined using the Folin-Ciocalteu reagent, and antimicrobial activities were determined using the agar well-diffusion method. *Euphorbia graminea* extracts had a low scavenging capacity, with an IC50 of 992.83 ± 0.44 µg/mL, while *Tetracera scandens* had a high scavenging ability, with an IC50 of 67.64 ± 0.66 µg/mL, compared to ascorbic acid, which had an IC50 of 11.41µg/mL. Total Phenolic Contents in *E. graminea* and *T. scandens* extracts ranged from 231.16±0.03 to 141.26 ± 0.02 mgGAE/g, respectively. *Euphorbia graminea* showed good antibacterial activity against *Staphylococcus aureus* with a MIC of 6.25 mg/mL while *T. scandens* showed good antimicrobial activity against *Candida albican* with a MIC of 6.25 mg/mL. Extracts from the two plants had high to moderate antibacterial and antioxidant properties. Therefore, these two plants can serve as source of antimicrobial and antioxidant agents.

Key Words: Antimicrobial, Antioxidant, Pathogenic-infections, *Euphorbia graminea*, *Tetracera scandens*

INTRODUCTION

Plant-based medicines are commonly prescribed for the treatment of infectious disorders by traditional medical practitioners all over the world (Komakech *et al*., 2017). Infectious diseases caused by multidrug-resistant microbes have emerged as a major public health issue, posing a threat to commonly prescribed drugs (Buoso *et al*., 2022) resulting in a variety of ailments, an increase in free radicals, and potentially life-threatening diseases and disability in humans. It has been estimated that about a quarter of the population becomes infected at least twice a year (Shuman and Malani, 2018). As a result, oxidative stress plays a key role in infectious illnesses. Progression of infection-related diseases can be associated with oxidative stress, and the production of reactive species during infection can potentially be lethal. Myeloperoxidase, NADPH oxidase, nitric oxide synthase, xanthine oxidase, and certain transition metals all produce reactive species during infection (Azizan *et al*., 2013). Tawadrous *et al*. demonstrated that oxidative stress is responsible for iron overload in hepatitis C infected individuals when reactive species are produced to any degree during infection (Tawadrous *et al*., 2012).

Antioxidant production is one of the biological methods for combating oxidative stress. An antioxidant is a chemical that can protect biomolecules and disease pathogens from oxidative damage by avoiding, decreasing, or restoring it (Wu *et al*., 2013). Antioxidants like vitamin E and melatonin have been shown by several authors to help therapy in situations of severe illnesses. According to Zhang *et al*., activation of myeloperoxidase caused sepsis in the myocardium, whereas vitamin E restored cardiac function. More specifically, another antioxidant, melatonin, has been shown to help in sepsis (Zhang *et al*., 2010, Salamone and Correa, 2012). As a result, it is critical to produce extremely efficient medications to combat multidrug-resistant bacteria and oxidative stress. Treat with antimicrobial agents and antioxidants might be effective in Oxidative stress can cause alleviating the burden of infectious diseases.

Euphorbia graminea L. (Euphorbiaceae) is a perennial plant with milky juice that grows 30-80 cm tall. In traditional medicine, it has been used to treat asthma coughs, bronchial problems, dyspnea, and pneumonia (Ernst and Frisén, 2015). *Euphorbia graminea* Jacq. is a plant that was first noticed in Nigeria in the mid-2000s as a new species of the *Euphorbia genus* (Aigbokhan and Ekutu, 2012). The scarcity of data on

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E. graminea in the literature shows that it is a newcomer to Nigerian flora. Although the plant has not been widely used in Nigeria to treat a variety of ailments, its applications may be as unique as those of other *Euphorbia species*. Some *Euphorbia species* have been recognized for their medicinal properties, *E. tirucalli*, for example, has molluscicidal action (Gupta *et al*., 2013). Antimicrobial activity of *E. hirta*, antioxidant and antiviral properties of *E. spinidens* have been reported (Karimi and Taherzadeh, 2016). Ogbulie *et al*. (2007) studied the antibacterial activity and toxicological potentials of crude ethanolic extracts of *Euphorbia hirta* (Ogbulie *et al*., 2007). However, little is known about the antimicrobial properties of methanol extracts of *Euphorbia graminea*.

Tetracera scandens L. (Dilleniaceae) is a perenial shrub with stem reaching up to 30 m in height (Lee and Heo, 2009). The root and stem are used in the treatment of hepatitis, edema, gout; and the stem is utilized as an anti-diarrhea (Connolly *et al*., 2013), anti-diabetic (Bui-Xuan *et al*., 2010), anti-HIV (Lee *et al*., 2012), and xanthine oxidase inhibitor (Connolly *et al*., 2013). Finding new sources of safe, effective, and affordable single herbal preparations that can combat free radicals and mitigate the negative effects of microbes is critical. As a result, the goal of this study

was to look into the antioxidant and antibacterial properties of methanol leaf extracts from *Euphorbia graminea* L. and *Tetracera scandens* L.

MATERIALS AND METHODS

Plant collection and authentication: *Euphorbia graminea* leaves were obtained along University of Ibadan Botanical Garden Road, while *Tetracera scandens* leaves were collected in Ondo State's woodland. Plants were identified and certified at the Forest Herbarium Ibadan (FHI) in the Forestry Research Institute of Nigeria (FRIN) with voucher specimen numbers 109674 and 110055. After three weeks of air drying, the plant components were crushed into a coarse powder.

Preparation of extracts: Absolute methanol was used to extract 150 g of powdered leaf plant materials at room temperature for 72 hours using cold maceration. Extracts were concentrated in vacuum using a rotary evaporator and extracts were stored in the refrigerator (4°C) until used.

DPPH free radical scavenging assay: The antioxidant capacity of the plant extract was determined using a modified version of (Xie and Schaich, 2014) using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radicals scavenging experiment. The crude extract stock solutions were produced in methanol to obtain a concentration of 1 mg/mL. With methanol, serial dilutions were done to generate concentrations ranging from 1000 to 15.63 µg/mL. Positive controls included ascorbic acid at doses ranging from 50 to 0.781 g/mL, whereas negative controls included all reagents except the extract or reference medicines. One hundred microlitre (100 µL) extract or standard, 100 µL methanol, and 150 µL newly prepared DPPH solution (0.04 mg/mL) were used in the test. For 30 minutes, the mixture was incubated at room temperature in the dark. A 96-microwell plate was used to measure absorbance. Using a SPECTRA max – PLUS 384 UV – Vis Spectrophotometer, the change in color from deep violet to bright yellow was measured at 517 nm. The extract's ability to scavenge the DPPH radical was determined using the following equation:

Percentage (%) Inhibition/ scavenging ability = A – B/A × 100.

Where A = Absorbance of the negative control, B = Absorbance of the sample

The experiment was conducted in triplicate, and the results were expressed as mean ± SEM. The effective concentration of samples required to scavenge DPPH radical by 50% (IC50) was obtained.

Determination of total phenolic content: The Folin–Ciocalteu technique was used to determine the content of phenolics in the plant extracts (Siddiq *et al*., 2018). The reaction mixture was made by vigorously mixing 0.5 mL of each plant's methanol extract (1 mg/mL) with 2.5 mL of 10% Folin-reagent Ciocalteu's for 5 minutes, then adding 2.5 mL of 7.5% NaCO3. Concurrently, a blank solution containing 0.5 mL methanol, 2.5 mL 10% Folin Ciocalteu's reagent and 2.5 mL 7.5% NaCO3 was produced. After, the reaction mixture was incubated for 2 hours at room temperature (25-29 °C). Using a SPECTRA max - PLUS 384 UV -Vis Spectrophotometer. The absorbance of the combination was determined at 765 nm. The calibration curve was created using the same approach for the standard (gallic acid) at concentrations ranging from 50, to 5 g/mL. The concentration of phenolics (mg/mL) was estimated using the calibration curve based on the observed absorbance, and the phenolic content was represented in terms of gallic acid equivalent as GA/g of extract in 1 mg/mL. The experiment was repeated three times, with the findings reported as mean ± SEM.

Antimicrobial assay

Source of microbial strains and cultures preparation: The test microorganisms; bacteria and fungus were obtained from the Pharmaceutical Microbiology Laboratory Department of the University of Ibadan, Ibadan, Nigeria. Bacteria Strains: Staphylococcus aureus (ATCC 6571), Pseudomonas aeruginosa (ATCC 27853), Salmonella typhimurium (ATCC 14028), and Escherichia coli (ATCC 14028) were among the reference strains employed (ATCC 25925). Aspergillus niger and Candida albican were among the fungi utilized. SDA (Sucrose dextrose agar) and MHA (Mueller Hinton agar) media were prepared as directed by the manufacturer. For bacterial and fungal culture, a single colony of each organism was injected into 5 mL SDA and MHA medium, respectively. For both bacterial and fungal cultures, all microorganisms were sub-cultured from the original culture and incubated overnight at 37 o C for 24 hours and 25 o C for 48 hours, respectively.

Antimicrobial drugs: The standard drugs, 0.1% (10 µg/mL) Gentamicin (bacteria) and 10 µg/mL Fluconazole (fungi) were used as the positive controls.

Antimicrobial assay: The test was carried out in accordance with method described by Jimoh and co-workers (Jimoh *et al*., 2020), The suspensions of bacteria and fungi cells (108 cfu / mL) were uniformly streaked on solidified nutrient and dextrox agar plates. A volume of 100 µL from each of the extract working concentrations serially diluted (6.25-100 mg / mL) was inoculated into wells (6 mm in diameter) already prepared. The experiment was carried out in three (3) replicates. Gentamicin (10 µg/mL) and ketoconazole (10

µg/mL) were employed as positive controls for bacteria and fungi while 0.1% methanol was utilized as a negative control.

Determination of minimum inhibitory concentration (MIC): The (MIC) of the extracts against test organisms was carried out adopting the method of Sonibare *et al*., 2016). Stock solution of extracts and agar to a concentration of 100 mg/mL. Serially, two fold dilutions of working concentration were made into petri-dishes (1.56 mg/mL, 3.13 mg/mL, 6.25 mg/mL, 12.5 mg/mL, 25 mg/mL, and 50 mg/mL) and left on the bench for about two hours to set. Different organisms were then inoculated on the different plates with different concentrations. The bacteria plates were incubated at 370C for 24 hours while that of fungal plates was incubated at 260C for 24-48 hours. The first plate in the above series with no sign of visible growth was reported as the minimum inhibitory concentration.

Statistical analysis: Experimental results were the mean±SEM of three measurements. The nonlinear regression program GraphPad Prism 5.0 ® version was used to obtain the inhibition concentration at 50 % (IC50) for the antioxidant study. The MIC was determined non statistically by the lowest concentration of extracts at which the microbes did not have visible growth.

RESULTS

The antioxidant activity of the extracts (DPPH, free radical scavenging activity) shows that *Tetracera scandens* has a better antioxidant activity with IC50 (67.64±0.66 µg/mL) than *Euphorbia graminea* with IC50 (992.83±0.44 µg/mL) when compared to the standard (Ascorbic acid) with IC50 (11.41±0.34 µg/mL). The phenolic content of a sample is evaluated by the quantity of phenol present in 1g of the gallic acid equivalent of the sample. As indicated in the table below, the amount of phenol content presented in mg Gallic acid Equivalent/g demonstrates that *Euphorbia graminea* has more phenolic content with (231.16±0.03 mg GAE/g) than *Tetracera scandens* with (141.26±0.02 mg GAE/g). (Table 1).

Table 1:
DPPH, free radical scavenging activity and Total Phenolic Content (TPC) of *Euphorbia graminea* and *Tetracera scandens* methanol extracts.

SAMPLE	IC_{50} (µg/mL)	TPC (mg GAE/g)
Euphorbia graminea	992.83± 0.44*	231.16±0.03$^{\#}$
Tetracera scandens	67.64 ± 0.66*	141.26±0.02
Ascorbic Acid	11.41±0.34	-

*P<0.05 - Ascorbic acid vs Plant extracts, $^{\#}$ *Euphorbia graminea vs Tetracera scandens*

Table 4:
Minimum inhibitory concentration (MIC) of the plant extracts on tests organisms

		Concentrations (mg/mL)/Zones of inhibitions (mm)					
Org.	**Extract.**	**50**	**25**	**12.5**	**6.25**	**3.12**	**1.65**
S. aureus	*E. graminea*	-	-	-	+	+	+
	T. scandens	-	+	+	+	+	+
P. aeruginosa	*E. graminea*	-	-	+	+	+	+
	T. scandens	-	+	+	+	+	+
S. typhimurium	*E. graminea*	-	-	+	+	+	+
	T. scandens	-	-	+	+	+	+
E. coli	*E. graminea*	-	-	+	+	+	+
	T. scandens	-	+	+	+	+	+
A. niger	*E. graminea*	+	+	+	+	+	+
	T. scandens	-	+	+	+	+	+
C. albican	*E. graminea*	-	+	+	+	+	+
	T. scandens	-	-	-	+	+	+

Key: - = +: growth; -: No growth
Bacteria strains: *S. aureus = Staphylococcus aureus*; *P. aeruginosa = Pseudomonas aeruginosa*; *S. typhimurium = Salmonella typhimurium*; *E. coli = Escherichia coli*;
Fungi strains: *A. niger = Aspergillus niger*; *C. albican = Candida albican*;

Table 3:
The effects of extracts on the test organisms

		Concentrations (mg/mL)/Zones of inhibitions (mm mean ±SEM)					
Org.	**Extract.**	**100**	**50**	**25**	**12.5**	**Gent. (10 µg/mL)**	**Fluconazole (10 µg/mL)**
S. aureus	*E. graminea*	21.32±0.32	20.66±0.00	18.66±0.03	16.00±0.33	20.66±0.02	-
	T. scandens	20.66±0.05	14.00±0.02	10.66±0.00	6.66±0.00		
P. aeruginosa	*E. graminea*	14.00±0.16	12.66±0.01	11.32±0.00	8.66±0.01	21.32±0.01	-
	T. scandens	16.66±0.37	10.00±0.00	10.00±0.66	6.00±0.033		
S. typhimurium	*E. graminea*	14.66±0.00	10.66±0.00	9.32±0.00	8.66±0.01	22.66±0.00	-
	T. scandens	26.00±0.01	21.32±0.01	13.32±0.00	9.32±0.01		
E. coli	*E. graminea*	14.66±0.03	10.66±0.01	9.32±0.03	8.66±0.01	28.00±0.01	-
	T. scandens	25.32±0.11	20.00±0.02	9.32±0.01	6.00±0.23		
A. niger	*E. graminea*	12.66±0.00	9.32±0.01	6.00±0.16	6.00±0.01	-	35.32±0.02
	T. scandens	32.00±0.00	14.660.00	10.00±0.01	5.32±0.00		
C. albican	*E. graminea*	35.32±0.03	20.00±0.02	14.00±0.03	6.66±0.00	-	33.32±0.02
	T. scandens	34.00±0.00	18.00±0.33	15.32±0.33	10.00±.00		

Bacteria strains: *S. aureus = Staphylococcus aureus*; *P. aeruginosa = Pseudomonas aeruginosa*; *S. typhimurium = Salmonella typhimurium*; *E. coli = Escherichia coli*; Control = Gentamicin
Fungi strains: *A. niger = Aspergillus niger*; *C. albican = Candida albican*; Control: Fluconazole

At 100 mg/mL, *E. graminea* extract showed inhibition zone of (35.32 mm) against *C. albican* and (21.32 mm) zone of inhibition against *S. aureus*, respectively, while *T. scandens* showed (32.00, 34.00) mm zone of inhibition against the strains of the fungi (*A. niger* and *C. albican*) respectively when compared to fluconazole and gentamicin at 10 µg/mL each.

The lowest concentration at which growth could be seen was used to calculate the extracts' Minimum Inhibition Concentration (MICs) as displayed in Table 4 below. *Euphorbia graminea* displayed MIC of 6.25 mg/mL against *Staphylococcus aureus*, while *T. scandens* demonstrated antifungal activity against *Candida albican* with a MIC of 6.25 mg/mL. Table 4.

DISCUSSION

The hunt for novel antimicrobial medications has intensified due to their potential use in the treatment of several infectious diseases. Furthermore, the emergence of resistant strains, mostly through the expression of resistance genes, has rendered a number of antimicrobial medications ineffective (Acheampong *et al*., 2010). Natural antioxidants have been explored intensively for decades in the hopes of discovering molecules that can protect against a variety of illnesses caused by oxidative stress and free radical damage. Many plants have been claimed to provide health benefits, such as antioxidant capabilities, to date (Cragg and Newman, 2018).

In this study, *Euphorbia graminea* exhibited a lower antioxidant activity compared to mild antioxidant activity of Tetracera scandens. Euphorbia graminea, on the other hand, had a higher phenolic content than Tetracera scandens. Ikpefan *et al*. (2021) reported 92.22 ± 1.92 mg/GAE/g in aqueous extract of *E. graminea* which was found lower than the amount in methanol extract of *E. graminea* (231.16±0.03 mg/GAE/g) in this investigation. Natural antioxidants, especially phenolic compounds can give electrons to oxidative molecules (Santos-Sánchez *et al*., 2019). According to Santos-Sánchez and colleagues, the degree of phenolic content determines the level of antioxidant capabilities (Santos-Sánchez *et al*., 2019) and the phenylpropanoids are the major category of plant secondary metabolites that are thought to act as antioxidants in nature (Kumar and Goel, 2019). DPPH free radical scavenging activity of aqueous extract of *E. graminea* in a previous study by Ikpefan *et al*. (2021) was six folds more active than the methanol extract of the *E. graminea* in this

investigation. The discrepancy in results might be related to the different extraction solvents utilized. The methanol extract of T. scandens had very significant antioxidant activity, with an IC50 value of 67.82 µg/mL, according to a prior publication (Soleha, 2020). Soleha's report on T. scandens significant antioxidant activity corroborated the findings of this investigation.

The antimicrobial activity of the methanol extracts of *E. graminea* and *T. scandens* on all test organisms examined varied in comparison to the standards. It was observed that the higher inhibition zones by the extracts could be as a result of the high concentrations of the active secondary metabolites and probably increase in the diffusing capacity of the extracts into the cells of the organisms, this was in accordance with a report by (Mills-Robertson *et al*., 2012) that increase in concentration could result in better antimicrobial activity of an extract. The lower MIC range for *S. aureus* in the extract of *E. graminea* and for *C. albican* in the extract of T. scandens recorded as show in Table 4 suggests the presence of a potential antimicrobial agent making it possible to reduce the growth of the bacteria and fungi organisms, thus, this extract might be explored for the development of antimicrobial agents. Progression of infectious disorders can promote increased oxidative stress and the formation of reactive species (Azizan *et al*., 2013). When reactive species produced during infection are discharged to any extent, they can have devastating repercussions for the illness (Tawadrous *et al*., 2012). Antioxidant production is one of the biological methods for combating the consequences of oxidative stress. Both plants extracts had antibacterial activity ranging from moderate to high. It is likely that the antimicrobial properties of the plant extracts were boosted by their ability to scavenge free radicals and high phenolic content. Antioxidants have been shown to reduce the severity of infectious diseases (Zhang *et al*., 2010, Salamone and Correa, 2012).

CONCLUSION

The *Euphorbia graminea* and *Tetracera scandens* extracts showed considerable antimicrobial activities. The high to moderate antioxidant activity of both plant extracts could be associated with their reasonable antimicrobial activities. These findings justified the plants as potential source of antimicrobial drugs and antioxidants. Isolation and characterization of active principles responsible for bioactivities will be emphasized in further research.

Acknowledgement

The University of Ibadan's Pharmaceutical Microbiology Laboratory Department and Pharmacognosy Laboratory Department provided the microorganisms and the lab space for this work, and the authors are grateful for their assistance

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