



IN VITRO ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF METHANOL LEAVES EXTRACTS OF *EMILIA ABYSSINICA* (SCH. BIP. EX A. RICH) C. JEFFREY (COMPOSITAE) AND *CISSAMPELOS OWARIENSIS* P. BEAUV. EX DC. (MENISPERMACEAE)

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ABSTRACT

Background: *Emilia abyssinica* and *Cissampelos owariensis* are medicinal plants used traditionally for the treatment of wounds. It is important to identify potential natural remedies for diseases caused by pathogenic microorganisms and oxidative stress. This study aimed to evaluate the antibacterial and antioxidant activities of *Emilia abyssinica* and *Cissampelos owariensis* leaves.

Methodologies: The phytochemical analysis was carried out using standard protocols. Methanol crude extracts from leaves of these plants were tested against various bacterial strains using the agar diffusion and agar dilution methods. The antioxidant potential of the extracts was evaluated using DPPH free radical scavenging assay. Total Flavonoid and Phenolic Compounds Content were quantified using Aluminium chloride test and Folin-Ciocalteu method respectively. The assays were done in triplicate and data were expressed as mean \pm SEM.

Results: The phytochemical analysis of these plants revealed the presence of saponins, flavonoids, tannins, alkaloids, and sterols. The results indicated that both plants exhibited remarkable antibacterial and antioxidant activities. *Emilia abyssinica* extract had lower MIC values for all the isolates as compared to *Cissampelos owariensis* extract. The DPPH antioxidant activity of both plants showed comparable percentage inhibition with ascorbic acid the standard used (EA – 78.2%; CO – 78.5%; AA – 80.3%). The Total Flavonoid Content for *Emilia abyssinica* and *Cissampelos owariensis* methanol crude extracts were 204.3 $\mu\text{g/mL}$ (Rutin equivalent) and 291.3 $\mu\text{g/mL}$ (Rutin equivalent) while Total Phenolic Compounds Content gave 37.6 $\mu\text{g/mL}$ (GAE) and 38.8 $\mu\text{g/mL}$ (GAE) respectively.

Conclusion: These findings suggest that *Emilia abyssinica* and *Cissampelos owariensis* leaves extracts have promising antibacterial and antioxidant properties, and further studies are needed to identify and isolate the bioactive compounds responsible for these activities.

Keywords: Antimicrobial, Antioxidants, *Cissampelos owariensis*, DPPH, *Emilia abyssinica*

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INTRODUCTION

Bacteria are single-celled organisms that are found in soil, water, and in the human body. They can either be beneficial or harmful, depending on the species. Some bacteria help to break down organic matter, while others cause infections and diseases in humans and animals [1]. These pathogenic bacteria can enter the human body through various routes, including ingestion, inhalation, or physical contact [2]. Once inside the body, they cause a wide range of infections, from mild skin infections to life-threatening systemic infections [3]. Plant-derived chemicals are a wide group of chemical compounds that have been found naturally in plants. The extensive existence of these compounds in antimicrobial agents, natural or synthetic substances that kill or inhibit the growth of microorganisms like bacteria and fungi, have

demonstrated potent activities and numerous researchers have utilized natural products to combat bacterial resistance [4 - 6]. These compounds have exhibited promising results in overcoming the emergence of antibiotic resistance in bacterial pathogens [7]. They can restore the clinical application of older antibiotics by increasing their potency and consequently, avoid development of resistance [8].

Emilia abyssinica also called *Senecio abyssinicus* is a useful plant in tropical Africa, commonly called “Amunimuye” by the Yoruba speaking tribe in Nigeria [9 – 11]. The parts of the plant mostly used are leaves, stems, and flowers. The plant is believed to possess potent medicinal properties in ethno-medicine [12]. It is an annual herb that grows in open places to heights of about 50 cm and found in lowlands and mountain elevations in North and Southern Nigeria,

and West Cameroon. It is widely distributed in central and east tropical Africa. *Emilia abyssinica* is used for various medicinal purposes by traditional healers in the treatment of stomach-ache, dysentery, fever, and wounds [11]. The leaves of the plant are boiled in water and the resulting infusion is taken orally as a remedy for stomach-ache and dysentery. It is also used as a purgative and for the treatment of rheumatism, scabies, and skin infections [13]. *Senecio abyssinicus* is a plant species that has been studied for its phytochemical constituents. The plant contains a variety of secondary metabolites, including alkaloids, flavonoids, saponins and terpenoids [14].

Cissampelos owariensis is commonly called velvet leaves, “ewe jenjoko or jokoje” among the Yoruba people and “Damal gwaraajii” in Hausa, Nigeria. The plant is a perennial climbing plant with annual stems that scramble over the ground or twine into the surrounding vegetation for support. It is found mostly in African countries like Nigeria, Sierra Leone, Congo, Tanzania and Angola [15]. *Cissampelos owariensis* is commonly used to treat gastrointestinal disorders such as diarrhea, dysentery, and stomach pain [16]. The plant's leaves are boiled and the decoction is consumed. It is used traditionally for various healing properties such as purgative, fertility, wound healing. In addition, the aerial parts of the plant have been used in the management of mental disorder; juice from the leaf is squeezed and given to patients experiencing psychosis in Nigeria [17]. Phytochemicals found in *Cissampelos owariensis* leaves are flavonoids, saponins, tannins and moderate amounts of alkaloids [18], two sesquiterpenes, which are bis (2- methoxy ethyl) phthalate and hexahydro 1,3-dimethyl (-4-phenyl-1H-azepine-4-carboxylic acid have been isolated from this plant [19]. The phytochemical investigation of the root and leaf extracts of *C. owariensis* yielded bisbenzylisoquinoline, isochrodrondendrin, berberine and cycleanine alkaloids [20].

The fascinating reports about the trado-medical properties of these plants, along with its wide range of reported biological activities, inspired us to delve into the leaves of both plants. Hence, the aim is to investigate the antibacterial and antioxidant properties of the methanol leaves extract of *Emilia abyssinicus* and *Cissampelos owariensis*.

MATERIALS AND METHODS

Chemicals

The chemicals and reagents used include; Ammonia solution 10%, Chloroform, Concentrated sulphuric acid, Dragendorff's reagent, Ferric chloride, Glacial acetic acid, Hydrochloric acid, Lead acetate, Mayer's reagent, Methanol, Methylene chloride, Olive oil, Sodium hydroxide, Wagner's reagent, Ethanol, Folin-Ciocalteu

reagent (Loba Chemie Pvt. Ltd), Sodium carbonate, Distilled water, Gallic acid (Molychem), Aluminium chloride (Loba Chemie Pvt. Ltd), Potassium acetate (Loba Chemie Pvt. Ltd.), Rutin (Loba Chemie Pvt. Ltd), Ascorbic acid, Gentamicin, and Mueller Hinton agar.

Collection of materials

The leaves of *Emilia abyssinica* and *Cissampelos owariensis* were collected in Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State during the month of April 2022. Contaminants were removed by hand picking from the bulk, pest-infested specimen were removed, and the leaves were washed with water to remove sand and other debris. The plants were identified and authenticated at Forest Herbarium Ibadan, Ibadan, Nigeria, with FHI number 113719 and 113715 respectively.

Preparation and extraction of plant samples

Both plants were collected, cleaned, and air-dried for a period of one month. The dried leaves were further dried using an oven for 30 minutes at a temperature of 40°C to remove all moisture contents. It was then pulverized. About 50 g of the pulverized sample of each plant was macerated in methanol in a ratio of 1:3 (pulverized leaves: methanol) in a container. The mixture was macerated for 72 hours at room temperature and stirred intermittently for optimal extraction of bioactive compounds. After 72 hours, the macerated samples were filtered using filter paper to remove solid particles. The filtrates were then concentrated under vacuo using a rotary evaporator set at 50°C. Further drying was done using a regulated water bath at 50°C to obtain dried crude extracts [21].

Phytochemical screening

Phytochemical screening of the pulverized leaves of *Emilia abyssinica* and *Cissampelos owariensis* were carried out using standard procedures [22, 23].

Evaluation of antibacterial activity

Test microorganisms

Ten clinical isolates including three *Staphylococcus aureus*, two *Pseudomonas aeruginosa*, two *Klebsiella pneumoniae* and one each of *Proteus mirabilis*, *Escherichia coli* and *Salmonella typhi* were obtained from Pharmaceutical Microbiology Laboratory, Olabisi Onabanjo University Sagamu, Ogun State while the typed strains of *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* NCTC 6571, *Klebsiella pneumoniae* ATCC 700303, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 700728 and *Salmonella typhi* ATCC 14028 were obtained from the Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Ibadan.

Extract standardization

The extracts were reconstituted with 50% methanol. Serial dilutions were carried out to obtain four different concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL.

Inocula standardization

Mueller-Hinton Agar (MHA) served as a medium for sub-culturing the isolates on sterile plates. The plates were incubated at 37°C for 24 hours. The broth cultures of the isolates were adjusted to the exact turbidity equivalent to 0.5 McFarland Standard as recommended by Clinical and Laboratory Standard Institute (CLSI).

Antibacterial susceptibility testing

The antibacterial activity of the extracts was determined using agar well diffusion assay method [24, 25]. Mueller Hinton Agar (MHA) was prepared as recommended by the manufacturer and allowed to cool to 50°C before pouring into pre-labeled sterile petri plates. Overnight culture of the test organism adjusted to 0.5 McFarland turbidity standard was used to inoculate the surface of solidified Mueller Hinton agar plate using a sterile swab stick. A sterile cork borer (9 mm) was used to punch six wells at equidistant wells on the Mueller Hinton Agar plates. Wells 1 – 4 contained four different concentrations of each extract (12.5 mg/mL – 100 mg/mL). Wells nine and ten were filled with 50% methanol and 20 µg/mL gentamicin, respectively. The plates were left on the bench for 45 minutes allowing for proper pre-diffusion of the extracts into the agar and incubated at 37°C for 24 hours. Subsequently, antibacterial activity was determined by measuring the diameter of zones of inhibition in millimeters.

Determination of minimum inhibitory concentration (MIC)

Using the agar dilution method, different concentrations of plant extracts were prepared by adding 2 mL of stock concentration of each plant extract to 18 mL of molten Mueller Hinton agar, cooled down to 45°C to obtain the desired final concentrations of 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.125 mg/mL, 1.563 mg/mL and 0.781 mg/mL. Bacterial suspensions adjusted to 10⁵ CFU/mL were used to inoculate the MHA plates containing different concentrations of the plant extracts. The plates were incubated at 37°C for 24 hours. The MIC was determined as the lowest concentration of plant extract in solid media where no growth was observed after 24 or 48 h [26].

Antioxidant activity evaluation

DPPH radical scavenging assay

The DPPH radical solution was prepared by dissolving 2.5 mg of DPPH in 100 mL of methanol (0.025 g/L). Different concentrations of *Emilia abyssinica* and

Cissampelos owariensis leaf extracts obtained from stock extract solution (1 mg/mL) were added to 3.9 mL of DPPH solution and the reactants incubated at 25°C room temperature for 30 minutes in a dark environment. In place of an extract, the ascorbic acid solution (a positive control) was used as standard. The mixture was shaken, and the solution was allowed to stand in the dark at room temperature for 30 minutes. Free radical scavenging activity was calculated from the absorbance values at 517 nm. Blank (methanol) was also measured and recorded [27].

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

Total phenol content estimation

The Total Phenol Content (TPC) of *Emilia abyssinica* and *Cissampelos owariensis* methanol leaves extracts were determined by spectrophotometric method using the Folin-Ciocalteu method, gallic acid was set as standard curve. 0.25 mL of each extract was mixed with 0.5 mL Folin-Ciocalteu phenol reagent. After 5 min, 5 mL of 7% Na₂CO₃ solution was added to the mixture, followed by the addition of 65 mL distilled water. The mixture was thoroughly mixed, allowed 90 min incubation at 25°C and the absorbance was read at 765 nm. The TPC value of the leaves crude extract (1 mg/mL) was calculated from the gallic acid standard curve expressed as GAE mL/g of extract. Blank was distilled water and the values were in triplicates [28]. The various concentrations of gallic acid used for the standard curve were 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.6 µg/mL, 7.8 µg/mL and 3.91 µg/mL.

Total flavonoid content estimation

Total Flavonoid Content (TFC) was determined using Aluminium chloride method [29]. One mL of sample (1 mg/mL) was mixed with 3 mL of methanol, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1 M potassium acetate and 5.6 mL of distilled water and kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420 nm using UV-Visible spectrophotometer. The total flavonoid content was determined from standard rutin calibration curve (0-100 µg/mL in methanol). The concentration of total flavonoid was expressed as mg of rutin equivalent/g of plant extract. The concentrations of rutin used for the standard curve were; 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.6 µg/mL and 7.8 µg/mL.

RESULTS AND DISCUSSION

Phytochemical screening

The qualitative phytochemical screening for the presence of different classes of metabolites in *Emilia abyssinica* and *Cissampelos owariensis* leaves indicated presence of saponins, tannins, flavonoids, alkaloids, steroids, and cardiac glycosides, but absence of cyanogenic glycosides, anthraquinones and amino acids in pulverized leaves of both plants (Table 1). Egharevba, et al. [30], reported that the leaves extract of *Senecio abyssinicus* showed presence of polyphenols, steroids, terpenoids, alkaloids, and cardiac glycosides. In addition, *C. owariensis* root methanol extract was found to contain flavonoids, alkaloids, resins, tannins, balsams, steroids, saponins, terpenes, cardiac glycosides, carbohydrate and phenols [31]. These previous studies supported the result of our study.

Table 1: Preliminary phytochemical screening of *Emilia abyssinica* and *Cissampelos owariensis* pulverized leaves

TEST	<i>E. abyssinica</i>	<i>C. owariensis</i>
Anthraquinones	-	-
Flavonoids	+	+
Cyanogenetic	-	-
Saponins	+	+
Sterols	+	+
Amino acids	-	-
Alkaloids	+	+
Tannins	+	+

Key: + Present, - Absent

Antibacterial activity of *Emilia abyssinica* and *Cissampelos owariensis* leaves extracts

Antimicrobial Sensitivity Testing involves testing the sensitivity of microorganisms to various concentrations of plant extracts. The microorganisms were cultured in the presence of different concentrations of the extract, and the results were evaluated based on the degree of growth inhibition [32]. Furthermore, Minimum Inhibitory Concentration (MIC) testing is a quantitative test that determines the minimum concentration of plant extract required to inhibit completely the growth of microorganisms using agar dilution method [33]. It is a valuable tool for identifying the most effective concentration of plant extract against a specific microorganism.

Emilia abyssinica methanol extract exerts antibacterial activity against some clinical bacterial isolates at used concentrations (Table 2) with zones of inhibition ranging from 10 to 20 mm while the extract did not show any activity on the following clinical isolates: *K. pneumoniae* 112, *P. mirabilis*, *S. aureus* 02, *E. coli* 71, *P. aeruginosa* 31 and *P. aeruginosa* 81. All the clinical isolates were not susceptible to the extract at 12.5 and 25 mg/mL. Higher antibacterial activity was observed against the typed strains than the clinical isolates. Only *K. pneumoniae* ATCC 700303 and *E. coli* ATCC 700728 had zones of inhibition at lower concentration of 12.5 mg/mL (Table 2). MIC values ranged between 0.78 – 1.56 mg/mL and 1.56 – 3.13 mg/mL among the clinical isolates and typed strains, respectively (Table 3).

Table 2: Antibacterial activity of *Emilia abyssinica* against clinical isolates and typed cultures (Zones of Inhibition in millimeters)

ORGANISMS	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	50% methanol + sterile water	Gentamycin
<i>Klebsiella pneumoniae</i> (112)	0	0	0	0	0	12mm
<i>Staphylococcus aureus</i> (8571)	11mm	0	0	0	0	24mm
<i>Salmonella typhi</i> (111)	12mm	11mm	0	0	0	16mm
<i>Proteus mirabilis</i>	0	0	0	0	0	20mm
<i>Staphylococcus aureus</i> (02)	0	0	0	0	0	15mm
<i>Escherichia coli</i> ATCC 25922	0	0	0	0	0	26mm
<i>Staphylococcus aureus</i> ATCC 29213	11mm	0	0	0	0	16mm
<i>Klebsiella pneumoniae</i> ATCC 700303	11mm	11mm	10mm	10mm	0	17mm
<i>Pseudomonas aeruginosa</i> ATCC 27853	12mm	12mm	13mm	0	0	22mm
<i>Escherichia coli</i> ATCC 700728	16mm	14mm	13mm	11mm	0	20mm
<i>Salmonella typhi</i> ATCC 14028	17mm	0	0	0	0	25mm
<i>Escherichia coli</i> (71)	0	0	0	0	0	0
<i>Klebsiella pneumoniae</i> (287)	10mm	10mm	0	0	0	22mm
<i>Staphylococcus aureus</i> (13)	20mm	15mm	0	0	0	23mm

<i>Pseudomonas aeruginosa</i> (31)	0	0	0	0	0	22mm
<i>Pseudomonas aeruginosa</i> (81)	0	0	0	0	0	0

Table 3: Total Minimal Inhibitory Concentration (MIC) of *Emilia abyssinica* leaves extract against different isolates

Test Organism	MIC (mg/mL)
<i>Staphylococcus aureus</i> (8571)	1.56
<i>Salmonella typhi</i> (111)	1.56
<i>Klebsiella pneumoniae</i> (287)	0.78
<i>Staphylococcus aureus</i> (13)	3.12
<i>Klebsiella pneumoniae</i> ATCC 700303	1.56
<i>Pseudomonas aeruginosa</i> ATCC 27853	1.56
<i>Escherichia coli</i> ATCC 700728	3.12
<i>Salmonella typhi</i> ATCC 14028	3.12

The crude methanol extract of *Cissampelos owariensis* were tested against different strains of microorganisms using agar dilution method [34]. The degree of growth inhibition was determined by measuring the diameter of the inhibition zone around the microbial growth. From the AST result in Table 4, the highest degree of activity was observed against *E. coli* ATCC 700728 (ZOI range of 12 – 14 mm) and *S. typhi* ATCC 14028 (ZOI of 13 mm). Moderate degree of activity was observed against *S. aureus* (13) and (02) (ZOI of 11 mm and 10 – 12 mm respectively), *S. aureus* (8571) (ZOI of 10 – 11 mm), *K. pneumoniae* (112), (287) and ATCC 700303 (ZOI of 10 mm, 10 mm and 10 – 12 mm, respectively), and *P. aeruginosa* (81) (ZOI of 11 mm). Strains of bacteria that showed no degree of activity include *S. aureus* ATCC 29213, *E. coli* (71), ATCC 25922, *P. aeruginosa* (31), ATCC 27833, and *S. typhi* (111). The test bacterial that were susceptible were further tested for the Minimum Inhibitory Concentration (MIC) to determine the lowest concentration of the extract. *Staphylococcus aureus* (8571), *Salmonella typhi* (111), *Staphylococcus aureus* (02), *Klebsiella pneumoniae* (287) gave an MIC value of 6.25 mg/mL while *Staphylococcus aureus* (13), *Pseudomonas aeruginosa* (31) and *Pseudomonas aeruginosa* (81) gave an MIC value of 12.5 mg/mL (Table 5).

The absence of growth inhibition zones using the agar well diffusion method may be caused by the active component in the plant extract not being able to

diffuse into the agar medium, as opposed to the low minimum inhibitory concentrations seen using the agar dilution method. According to Eloff [35], Agar diffusion experiments use an aqueous agar substrate, and many antimicrobial chemicals found in plant extracts are relatively non-polar, meaning that they do not diffuse well in this matrix.

Several researchers have documented the antimicrobial activity of *Emilia* species. Antibacterial activity of aqueous, ethanol and methanol extracts of *Emilia sonchifolia* was reported [36, 37]. Teke et al. [38] also reported the antidiarrheal effects of both methanol and aqueous extracts of the leaves of *Emilia coccinea*. To the best of our knowledge, this is the first report on the antibacterial activity of *Emilia abyssinica* leaves extract.

The antimicrobial activity of *Cissampelos* species has also been reported [39, 40]. Ngoci et al. [39], found that *Cissampelos pareira* exhibits antibacterial efficacy against both Gram-positive and Gram-negative bacteria. Crude methanol root extract of *Cissampelos owariensis* was reported to be active against *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Candida albicans* [41]. This study also reported lower MIC values against the typed bacterial strain and clinical bacterial isolates including *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Table 4: Antibacterial activity of *Cissampelos owariensis* against clinical isolates and typed cultures (zones of inhibition in millimeters)

Test organism	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	50% methanol + sterile water	Gentamycin
<i>Klebsiella pneumoniae</i> (112)	10mm	10mm	0	0	0	12mm
<i>Staphylococcus aureus</i> (8571)	11mm	10mm	10mm	0	0	23mm
<i>Salmonella typhi</i> (111)	0	0	0	0	0	25mm
<i>Proteus mirabilis</i>	0	0	0	0	0	0
<i>Staphylococcus aureus</i> (02)	12mm	11mm	10mm	11mm	0	13mm
<i>Escherichia coli</i> ATCC 25922	0	0	0	0	0	22mm
<i>Staphylococcus aureus</i> ATCC 29213	0	0	0	0	0	23mm
<i>Klebsiella pneumoniae</i> ATCC 700303	12mm	11mm	0	0	0	16mm
<i>Pseudomonas aeruginosa</i> ATCC 27853	0	0	0	0	0	22mm
<i>Escherichia coli</i> ATCC 700728	14mm	12mm	0	0	0	21mm
<i>Salmonella typhi</i> ATCC 14028	13mm	13mm	0	0	0	18mm
<i>Escherichia coli</i> (71)	0	0	0	0	0	17mm
<i>Klebsiella pneumoniae</i> (287)	10mm	0	0	0	0	25mm
<i>Staphylococcus aureus</i> (13)	11mm	0	0	0	0	20mm
<i>Pseudomonas aeruginosa</i> (31)	0	0	0	0	0	22mm
<i>Pseudomonas aeruginosa</i> (81)	11mm	0	0	0	0	0

Table 5: Minimum Inhibitory Concentration (MIC) of *Cissampelos owariensis* methanol leaves extract against different isolates

Test organisms	MIC (mg/mL)
<i>Staphylococcus aureus</i> (8571)	6.25
<i>Salmonella typhi</i> (111)	6.25
<i>Staphylococcus aureus</i> (02)	6.25
<i>Klebsiella pneumoniae</i> (287)	6.25
<i>Staphylococcus aureus</i> (13)	12.5
<i>Pseudomonas aeruginosa</i> (31)	12.5
<i>Pseudomonas aeruginosa</i> (81)	12.5
<i>Klebsiella pneumoniae</i> ATCC 700303	6.25
<i>Pseudomonas aeruginosa</i> ATCC 27853	6.25
<i>Escherichia coli</i> ATCC 700728	6.25
<i>Salmonella typhi</i> (14028)	6.25

Antioxidant assay

DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The colour changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition. The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant by their hydrogen donating ability. The electrons become paired off and solution loses color stoichiometrically depending on the number of electrons taken up [42]. The result of DPPH showed that *Emilia*

abyssinica and *Cissampelos owariensis* possess highest percentage inhibition of 78.17% and 78.51% at concentration of 1.0 mg/mL when compared to ascorbic acid standard with percentage inhibition of 80.28% at the same concentrations (Figure 1). This implies that both plants contain high amount of antioxidant principles that mop up free radicals in the body when used. Preceding studies confirmed the antioxidant property of the flower extract and fractions of *Senecio abyssinicus* leaves [14, 30]. Several species of *Senecio* have been reported to possess strong antioxidant property. *Senecio salsuginea* was reported to possess an inhibitory concentration (IC₅₀) of 26.23 µg/mL [43], and ethyl acetate fraction of *Senecio stabianus* showed an

IC₅₀ value of 35.5 mg/mL [44]. Furthermore, Omotoso *et al.* [45] reported that methanol extract of *C. owariensis* possesses abundant phenolic compounds, which explains its antioxidant property.

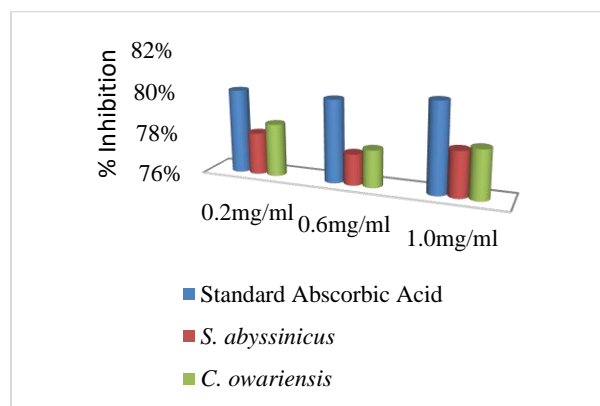


Figure 1: DPPH scavenging activity of methanol leaf extracts of *E. abyssinica* and *C. owariensis* with respect to standard Ascorbic acid

Phenolic compounds in plants are known to act as free radical scavengers and it has been opined that the antioxidant activity of most of the plant produce is mainly due to the presence of phenolic compounds [46, 47]. Basically, antioxidant mechanism of polyphenolic compounds is based on their hydrogen donating and metal ion chelating abilities [48]. The total phenol content is used to analyze tannins, anthocyanins, monomeric phenolic compounds and polymeric pigments present in a sample. A correlation between the phenolic content and antioxidant activity has been shown earlier by number of researchers [49, 50]. The amount of total phenol was investigated by Folin-Ciocalteu method [50]. The total phenol content of *Emilia abyssinica* and *Cissampelos owariensis* methanol leaf extracts (1 mg/mL) were 37.61 µg/mL and 38.78 µg/mL of gallic acid equivalent per gram of plant extract with reference to gallic acid standard curve.

Flavonoids are a group of naturally occurring compounds that have been shown to possess antioxidant activity, which makes them useful in various applications, including pharmaceuticals, food additives, and cosmetics [51]. The TFC assay involves the quantification of total flavonoids present in a plant sample. One of the most widely used methods for determining TFC is an aluminum chloride colorimetric assay. The potential antioxidant activity of flavonoid is associated with the chemical structures o-diphenolic group, a 2–3 double bond conjugated with the 4-oxo function and hydroxyl groups in positions 3 and 5 [52, 53]. Various concentrations of standard rutin solution were used to prepare a standard calibration curve [54]. The total flavonoid content of the methanol leaf extract

of *E. abyssinica* and *Cissampelos owariensis* (1 mg/mL) were 204.27 µg/mL and 291.28 µg/mL, respectively with reference to rutin standard curve. Comparatively, we discovered that *C. owariensis* have higher flavonoid content than *E. abyssinica*. The total flavonoid content of the methanolic extract of *Biophytum sensitivum* whole plant methanol extract was found to be 9.49 µg with respect to quercetin standard curve [55]. The antioxidant activity, total phenolic and flavonoid contents of whole plant extracts of *Torilis leptophylla* have also been reported by Saeed *et al.* [49]. The methanol leaves extract of *Costus afar* was reported to contain 3.13% of flavonoid and 0.77% of phenol respectively [56]. These previous reports support our results and confirm the presence of different quantity of flavonoids and phenolic compounds in medicinal plants.

Table 6: Concentration of phenolic compounds in methanol leaf extracts of *Emilia abyssinica* and *Cissampelos owariensis*

Plant samples	Concentration (µg/mL)
<i>Emilia abyssinica</i>	37.61
<i>Cissampelos owariensis</i>	38.78

Table 7: Concentration of flavonoid contents in methanol leaf extracts of *Emilia abyssinica* and *Cissampelos owariensis*

Plant sample	Concentration (µg/mL)
<i>Emilia abyssinica</i>	204.27
<i>Cissampelos owariensis</i>	291.28

CONCLUSION

These findings suggest that *Emilia abyssinica* and *Cissampelos owariensis* have promising antibacterial and antioxidant properties. The activities possessed by these plants are due to the phytoconstituents present. Further studies are warranted to identify and isolate bioactive compounds responsible for these activities.

ACKNOWLEDGEMENTS

The authors are grateful to Mr Adedokun and Mr Adeoti of the Pharmacognosy Department, Olabisi Onabanjo University, for their technical assistance during the bench work.

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