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Ushie OA
Department of Chemical Science, Federal University Wukari, Nigeria

Ama SO
Department of Chemical Science, Federal University Wukari, Nigeria

Aasegh TJ
Department of Chemical Science, Federal University Wukari, Nigeria

Nyikyaa JT
Department of Chemical Science, Federal University Wukari, Nigeria

Isemede A
Chemistry Department, Alvan Ikoku College of Education Owerri, Imo State

Edet EE
Department of Chemistry, University of Cross River State Calabar, Nigeria

Correspondence
M Raju
Tamil Nadu rice research institute, Tamil Nadu agricultural university, Aduthurai, Thanjavur, Tamil Nadu, India

Antioxidant activities of *Emilia sonchifolia* Chloroform, acetone and methanol leaf extracts

Ushie OA, Ama SO, Aasegh TJ, Nyikyaa JT, Isemede A and Edet EE

Abstract
Antioxidant activities of chloroform, acetone and methanol extracts of *Emilia sonchifolia* leaf extracts were evaluated using 1, 1-diphenyl-2-picrylhdrazyl (DPPH) radical scavenging assay in which all the leaf extract shows remarkable activities. The DPPH radical scavenging assay shows that extracts of methanol and acetone showed a good scavenging activity among all the extracts.

Keywords: Antioxidant activity, ascorbic acid, *Emilia sonchifolia* DPPH

Introduction
The use of plants in the management and treatment of diseases started with life. Many indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes (Okwu 2001) [7]. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Subhashini *et al*., 2010) [11]. Ojelere *et al*., (2014) [12] point out that medicinal plant are plants containing inherent active ingredients used to cure disease or relive pain.
Plants have a large number of bioactive compounds with high antioxidant activity which can be determined by the study of the antioxidant activity of different plant species which could contribute to revealing of the value of these species as a source of new antioxidant compounds (Chaves *et al*., 2020) [2]. Amid these molecules, the compounds derived from secondary metabolism, specifically phenolic compounds, play a primary role against oxidative stress (Pang *et al*., 2018) [8]. These compounds are known to act as antioxidants not only for their ability to donate hydrogen or electrons but also because they are stable radical intermediates (Ni´ciforovi *et al*., 2010) [5]. Phenolic compounds also have protective effects on humans when the plants are consumed as food (Ni´ciforovi *et al*., 2010) [5]. Generally, the antioxidant capacity of phenols in plant extracts is effective at low concentrations, and in humans, it is associated with the prevention of cardiovascular disease and cancer (Duthie *et al*., 2000) [3]. Hence, the determination of the antioxidant activity of the extract of different plant species could contribute to establishing the value of these species as a source of new antioxidant compounds (Miliauskas *et al*., 2004) [4].
Traditional medicines have some limitations such as lack of prescription and scientific proof. Some herbs look so common to the untrained eye that they are often mistaken for one another. The misclassification of species and the mistaken substitution is a real danger in the preparation and administration of herbal medicine. The plant kingdom holds many species of plants that contain substances of medicinal value which are yet to be discovered. *Emilia sonchifolia* has been used in traditional medicine for many years. This research gives an insight on the scientific data on the antioxidants activities of *Emilia sonchifolia*. The aim of this research is to study antioxidants activities of crude leaf extracts of *Emilia sonchifolia* in chloroform, acetone and methanol.

Materials and Methods
Sample Collection, Preparation and Extraction
The *Emilia sonchifolia* leaves were collected from their natural habitat in Calabar Municipality Local Government Area of Cross River State, Nigeria and were air dried for two weeks; the dried sample was chopped and grounded into fine powder. The extracts of the leaves were prepared by soaking 100 g of the sample in 250 ml chloroform for 72 hours with frequent agitation.

The resulting mixture was filtered by gravity filtration and the filtrate was concentrated by evaporation using rotatory evaporator, kept in a vacuum oven over night at room temperature to remove all the solvent and weighed. The procedure was repeated on the residue using acetone and methanol sequentially in order of polarity. The extracts were stored in a desiccator until required for testing.

Antioxidant Assay using DPPH Assay (2, 2-diphenyl-1-picrylhydrazyl)

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang *et al.*, (2001) [1] and Rahman *et al.*, (2016) [9]. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

Principle

2, 2- Diphenyl -1- Picryl Hydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

$$(DPPH) + (H\text{-}A) \longrightarrow DPPH\text{-}H + (A)$$

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Reagent preparation

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

Working procedure

Different volumes of the extract were taken and made up to 2ml with methanol. The following concentrations of the extract were tested (0.1, 0.3, 0.5, 0.7, and 1.0 mg/ml). Vitamin C was used as the antioxidant standard at concentrations (0.1, 0.3, 0.5, 0.7, and 1.0 mg/ml). 0.5ml of 1mM of DPPH in ethanol was added to each of the sample solutions. A blank solution was prepared containing the same amount of methanol and DPPH. The sample solutions are incubated in the dark for 3ominutes before reading the absorbance at 517nm. The radical scavenging activity was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A - B}{A} \times 100$$

Where

A = Absorption of the blank sample without extract.
B = Absorption of the extract.

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (Bilos, 1958). About 0.1mM of DPPH in ethanol was prepared and 1ml of this solution was added to 3.0 mL of extract solution in ethanol at different concentrations (5.0, 2.5, 1.25, 0.625 & 0.312 mg/mL). Thirty minutes later, the decrease or increase absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The same experiment was carried out on ascorbic acid which is known antioxidant. All test and analysis were run in triplicate and the results obtained were averaged.

Results and Discussions

Results

Results of the antioxidant activities of of chloroform, acetone and methanol extracts of *Emilia sonchifolia.*

Table 1: The antioxidant activities of of chloroform, ethyl acetate, acetone, methanol extracts of *Emilia sonchifolia* and vitamin C

Conc. (mg/ml)	Inhibition (%)			
	CE	AE	ME	Vitamin C
0.5	3.31	28.18	39.50	87.02
0.25	3.87	24.59	30.39	86.46
0.125	6.35	23.76	29.01	85.36
0.0625	7.46	23.20	27.07	84.25
0.03125	8.84	21.82	25.69	83.98

Discussion

To determined the antioxidant activity of a specific solution, there will be a significant decreased in the absorbance for sample which contain antioxidant compound (purple colour vanishing coupled with the yellow color build up clearly noticed by naked eye) the intensity of the yellow colour was directly proportional with the antioxidant activity in the tested solution, the higher scavenging indicate the higher activity (Sagare and Singh 2011) [10]. The free-radical scavenging activity was evaluated by accessing it's discolouration of 2,2-diphenyl-1-picrylhrozyl radical (DPPH) in methanol by a slightly modified method of Williams *et al*., 1995. The following concentrations of the extract were tested (0.5, 0.25, 0.125, 0.062 and 0.0313 mg/ml). The decrease in absorbance was monitored at 517nm. Vitamin C was used as the antioxidant standard at concentrations (0.5, 0.25, 0.125, 0.062 and 0.0313 mg/ml).

The crude chloroform extract of *Emilia sonchifolia* displayed inhibition of DPPH radical scavenging activity at the range of 28.18%, 24.59%,, 23.76%, 23.20% and 21.82% with the concentration of 0.5, 0.25, 0.125, 0.062 and 0.0313 mg/ml respectively while vitamin C showed minimum radical scavenging activity of 83.98% and maximum activity of 87.02% (Figure 2).

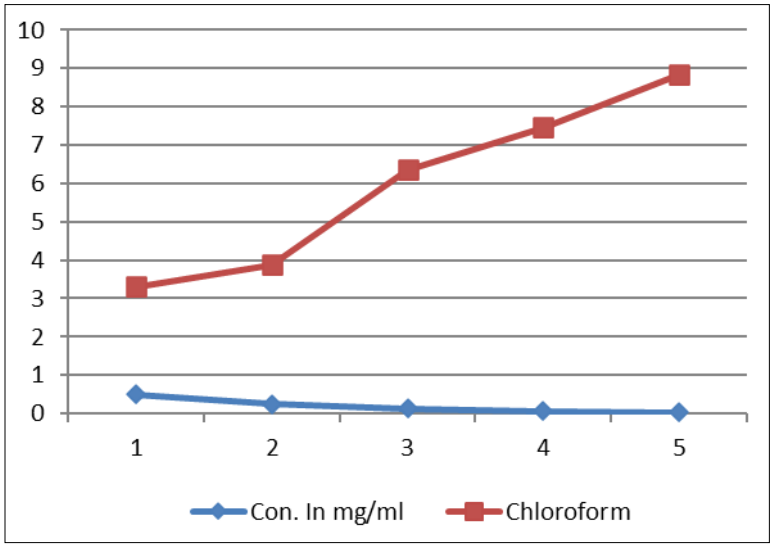

Fig 1: Plot of Concentration Vs % Inhibition for Chloroform extract

The crude acetone extract of *Emilia sonchifolia* displayed inhibition of DPPH radical scavenging activity at the range of 3.31%, 3.87%,, 6.35%, 7.46% and 8.84% with the concentration of 0.5, 0.25, 0.125, 0.062 and 0.0313 mg/ml respectively while vitamin C showed minimum radical scavenging activity of 83.98% and maximum activity of 87.02% (Figure 1)

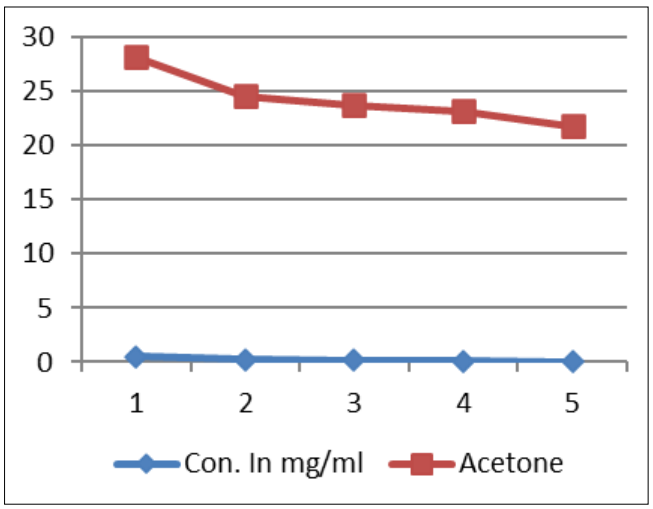

Fig 2: Plot of Concentration Vs % Inhibition for Acetone extract

The crude methanol extract of *Emilia sonchifolia* displayed inhibition of DPPH radical scavenging activity at the range of 39.50%, 30.39%, 29.01%, 27.07% and 25.69% with the concentration of 0.5, 0.25, 0.125, 0.062 and 0.0313 mg/ml respectively while vitamin C showed minimum radical scavenging activity of 83.98% and maximum activity of 87.02% (Figure 1)

Conclusion

In present study, chloroform, acetone and methanol extracts of *Emilia sonchifolia* were studied for their antioxidant capacity using DPPH radical scavenging assay. The DPPH radical scavenging assay shows that the extracts of methanol and acetone showed a good scavenging activity among all the extracts. The results obtained showed that this plant is very important from medicinal point of view and it needs further phytochemical exploitation to isolate phytochemical constituents showing antioxidant activity.

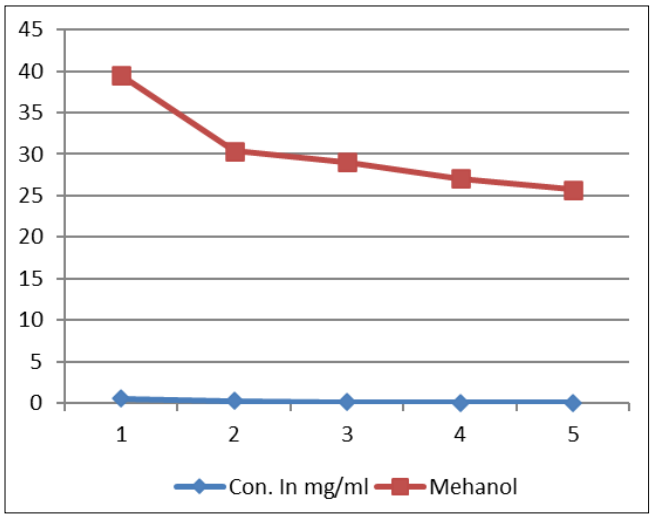

Fig 3: Plot of Concentration Vs % Inhibition for Methanol extract

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