

In-vitro Free Radical Scavenging Ability, Phytochemical Screening and Total Polyphenolic Contents of Different Extracts of *Emilia praetermissa* Leaves

Yemisi Rufina Alli Smith

Department of Biochemistry, Faculty of Science, Ekiti State University, Nigeria

Abstract : *Emilia praetermissa* is a herb that belongs to the family of *Asteraceae*. It is used as vegetable in many regions of West Africa and Central America. Many diseases/disorders are linked to oxidative stress due to generation of free radicals. The present study was undertaken to evaluate the antioxidant potentials of aqueous, ethanolic and methanolic extract of *Emilia praetermissa* leaves by investigating its phytochemicals constituents and *in-vitro* antioxidant potentials. Antioxidant activity of the extracts was screened for by measuring its total flavonoid content, total phenol content, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), hydrogen peroxide (H₂O₂), nitric oxide (NO), Hydroxyl (OH), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals scavenging activities, Iron chelation ability and ferric reducing property. The result showed that the aqueous extract is richer in phytochemical constituents than the other two extracts. Total phenol and Total flavonoids concentration were also higher in the aqueous extract. All the extracts scavenge free radicals in a concentration dependent manner with the aqueous extract show the most scavenging ability. The Free radical scavenging potentials of the extracts particularly the aqueous extract was found to be proportional to its polyphenolic contents. These findings revealed that the leaves of *Emilia praetermissa* contain biologically active constituents and therefore may be used as antioxidant supplements for the management of free radicals related diseases.

Keywords: *Emilia praetermissa*, free radicals, antioxidants, polyphenolics, diseases.

I. INTRODUCTION.

Plant-derived substance have become of great interest due to their versatile applications especially plant based constituents that can be derived from any part of a plant ranging from leaves to the roots[1]. The therapeutic properties of medicinal plants rely on the bioactive compounds which are also known as phytochemicals [2],[3]. Phytochemicals are naturally occurring chemicals in plants that confer a protective function to plants against bacteria, viruses, fungi, damage by free radicals, insects and

herbivores that feed on them and any other environmental threat[4]. Free radicals also known as reactive oxygen species (ROS) have a potential to damage cell components resulting to diseases. They are involved in initiation and progression of

many diseases among humans like cancer, cardiovascular diseases[5]. Free radicals play a dual role in the organism as both deleterious and beneficial factor. In low/moderate concentrations, free radicals or reactive oxygen species (ROS) are involved in normal physiological functions required for cellular signalling. However, excessive production of free radicals or a decrease in antioxidant levels leads to oxidative stress. Antioxidative phytochemicals present in medicinal plants such as flavonoids and phenolics participate in the management of diseases especially those caused by free radical damage by preventing the oxidative damage by free radicals[6]. *Emilia praetermissa* is an herb that belongs to the family of *Asteraceae*. *Emilia praetermissa* is commonly found in Nigeria, Congo, Burundi, Sudan, Kenya, Uganda, Tanzania, Malawi, Zambia, Angola, Zimbabwe and Mozambique. The use of *Emilia praetermissa* as a vegetable has been reported[7]. This study therefore focuses on, screening of the phytochemicals present in three extracts of *Emilia praetermissa* leaves and further evaluates the antioxidant activities of the extracts by determining its polyphenolic constituents and free radical scavenging potentials.

II. MATERIALS AND METHOD

A. Collection and Identification of Plant Sample

The *Emilia praetermissa* leaves were collected and identified by at the Herbarium section of Plant Science and Forestry Department, Ekiti State University, Ado-Ekiti (EKSU), Nigeria. A voucher specimen was deposited in the Departmental Herbarium the voucher No:UHAE-2017/105.

B. Sample Preparation and Extraction

The leaves were air dried and then pulverized into powder using an electric blender. Fifty grams of the powdered sample was extracted with 250 ml of 70% ethanol. The solvent was removed by filtration using a Buchner funnel with Whatman's No 1 filter paper. The filtrate was concentrated to dryness under vacuum in a rotary evaporator thereafter; the extract was collected in clean bottles and left opened in a laboratory fume hood for complete evaporation of residual solvent. The percentage yield for the extract was 4.4 % w/w.

C. Phytochemical Screening

Screening of *Emilia praetermissa* phytochemical constituents such as alkaloids, saponin, flavonoids, tannins, phenols, steroids, and terpenoids was carried out using the method of [8] with little modification from the method of [9].

D. Determination of Total Phenol Content

The total phenolic contents were determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method [10].

E. Determination of Total Flavonoid Content

Total flavonoid content was measured by the aluminium chloride colorimetric assay [11].

Determination of Ferric Reducing Ability

The ferric reducing capacity of the extracts was investigated by using the potassium ferricyanide-ferric chloride method [12].

F. Determination of Iron Chelation Ability

The Iron chelating capacity of *Emilia praetermissa* extracts was determined using the method described by [13].

Determination of DPPH Radical Scavenging Ability

The ability of aqueous extracts of *Emilia praetermissa* in scavenging the DPPH radical generated was estimated according to the method reported by [14] with slight modifications [15].

G. Determination of ABTS Radical Scavenging Ability

The antioxidant capacity was estimated in terms of the ABTS^{•+} radical scavenging activity following the procedure described by [16].

H. Determination of Nitric Oxide Radical Scavenging Ability

Nitric Oxide (NO) generated from sodium nitroprusside (SNP) was measured according to the method of [17].

I. Determination of Hydrogen Peroxide Radical Scavenging Ability

The ability of the *Emilia praetermissa* extracts to scavenge hydrogen peroxide was determined according to the method of [18].

J. Determination Hydroxyl Radical Scavenging Ability

The 2-deoxyribose assay was used to determine the scavenging effect of the extract on the $\cdot\text{OH}$ radical, as reported by [19] with minor modifications [15].

III. RESULT AND DISCUSSION

The results of this study confirm the presence of phytochemical constituents which are known to exhibit medicinal as well as physiological activities [20]. The phytochemical constituents of the three extracts of the leave of *Emilia praetermissa* indicated the presence of alkaloids,

flavonoids, phenols, terpenoids, steroids saponins and tannins as presented in Table 1 with an absence of saponins in the ethanolic and methanolic extract.

The major active nutraceutical ingredients in plants are flavonoids. A nutraceutical is any nontoxic food extract supplement that has scientifically proven health benefits for both the treatment and prevention of disease [21]. The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the leaves of the plants studied. The presence of terpenoids, saponins, tannins and phenolics suggests the reason why the leaves have anti-inflammatory activity as reported by [22]. The antifungal activity reported by [23] could be due to the presence of tannins while antibacterial activity due to the presence of steroids which are reported with that activity [24]. The leaves could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection.

The total Phenolics and total Flavonoid concentration of the extracts are presented in Table 2. The results show that the aqueous extract of *Emilia praetermissa* were richer in Phenolic and flavonoid than the other extracts. Natural antioxidants such as phenolic acids and flavonoid compounds from plants may offer resistance against the oxidative stress by scavenging free radicals, inhibiting lipid peroxidation, and by other mechanisms [25]. Antioxidant nutrients such as vitamin E, carotenoids, vitamin C and numerous polyphenolic compounds directly scavenge reactive oxygen species. This is why Antioxidant nutrients are theorized to constitute an endogenous defense against oxidative cells. The plant derived antioxidants have the tendency of contributing to the antioxidant activity and thus are able to play a vital role in the management of clinical disorders. Phenolic and flavonoids are the polyphenolic compounds which have been found to have free radical scavenging activity [26]. These compounds donate hydrogen molecules to free radicals and thus act as antioxidants. The results indicate that the aqueous extract has greater phenolic and flavonoid content than the methanol and ethanol extract but all extracts have the potential for antioxidant activity.

The free radical scavenging effects of the extracts of *Emilia praetermissa* are presented in Figure 1-5. The results showed a considerable free radical scavenging activity of different extracts in a dose dependent manner against DPPH, ABTS, hydrogen peroxide, nitric oxide and hydroxyl radicals when compared with control (Ascorbic Acid) at different concentrations. Aqueous extracts showed slightly higher scavenging activity than methanol and ethanol extracts in this study followed by the ethanolic extracts while the methanolic extracts showed the lowest scavenging ability. The iron chelation ability and the ferric reducing ability of the extracts are presented in Figure 6-7. All the different extracts were able to chelate iron and reduce ferric ion in a dose-dependent manner with the aqueous extract showing the greater ability. All the extracts showed a considerable free

radical scavenging activity when compared with control (Ascorbic Acid). Aqueous extracts showed slightly higher activity than the methanol and ethanol extracts. These results indicate that the *Emilia preatensis* have hydrogen donating ability to scavenge the free radicals. This radical scavenging ability of the extract could be linked to polyphenolic compounds present in the plant.

Ferric Reducing Ability is broadly used to estimate the antioxidant activity of polyphenols. It is associated with the presence of reductones which shows antioxidant actions by breaking the free radical chain and donating a hydrogen atom[27,29]. The reduced ability of a compound is related with its electron transfer ability and therefore they may serve as a significant indicator of its antioxidant activity[26,28].

Table I: Phytochemical Constituents of the Extracts of the Leaves *Emilia preatensis*

CONSTITUENTS	LEAVE EXTRACTS		
	Aqueous	Methanol	Ethanol
ALKALOIDS	+	+	+
FLAVONOIDS	+	+	+
PHENOLS	+	+	+
SAPONINS	+	-	-
STEROIDS	+	+	+
TANNINS	+	+	+
TERPENOIDS	+	+	+

+ means "present" and - means "Absent"

Table II: Polyphenolic Contents of Different Extract of *Emilia preatensis*.

EXTRACTS	TOTAL PHENOL (µg GAE/mg)	TOTAL FLAVONOID(µg QE/g)
AQUEOUS	3.28±0.15 ^a	2.96±0.15 ^a
METHANOL	1.97±0.15 ^b	2.63±0.13 ^{ab}
ETHANOL	2.92±0.13 ^c	2.17±0.16 ^b

Data are expressed as means ± SD (n=3). GAE and QE are Gallic acid and Quercetin Equivalent. Values with different superscript denotes significant differences at p<05.

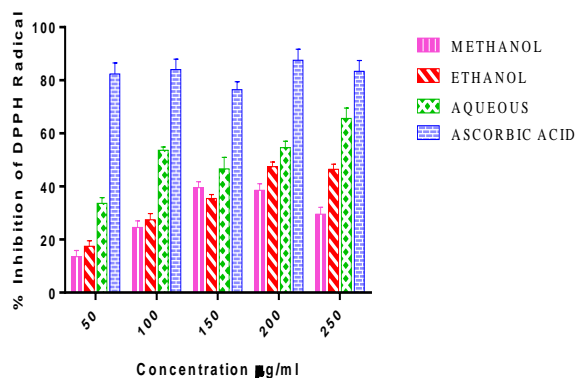


Figure 1: Scavenging Effects of Different Extract of *Emilia preatensis* Leaves on DPPH radical. The results are means ± SD (n=3).

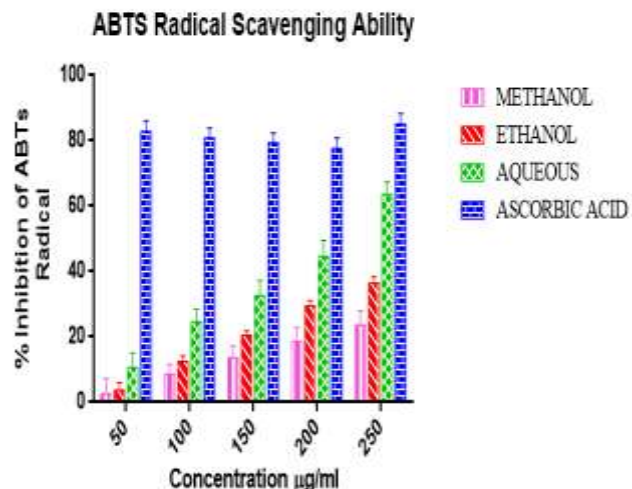


Figure 2: Scavenging effects of Different Extract of *Emilia preatensis* Leaves on ABTS radical. The results are means ± SD (n=3).

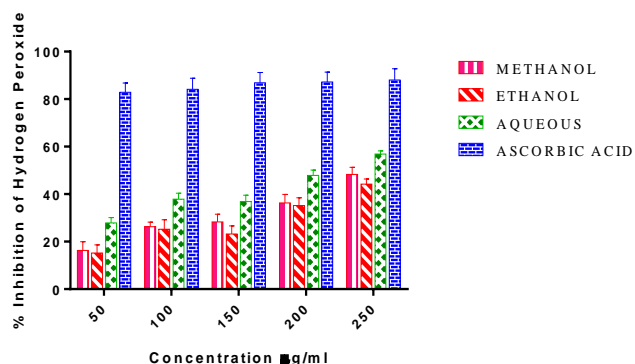


Figure 3: Scavenging Effects of Different Extract of *Emilia preatensis* leaves on Hydrogen Peroxide radical. The results are means ± SD (n=3).

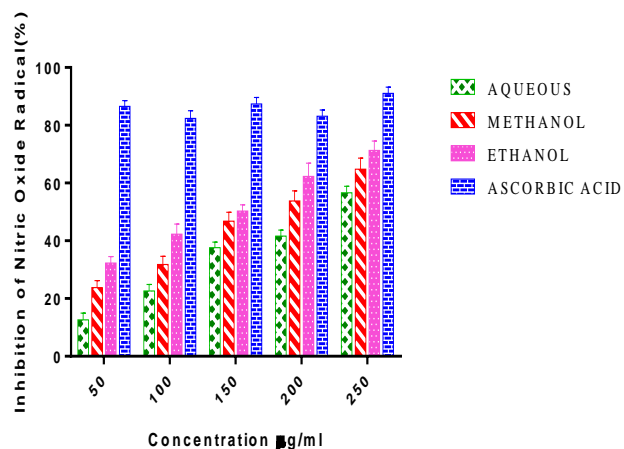


Figure 4: Scavenging effects of Different Extract of *Emilia preatensis* Leaves on Nitric Oxide radical. The results are means ± SD (n=3).

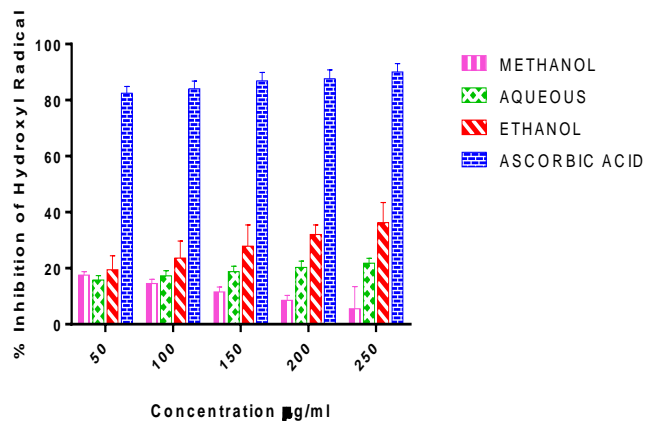


Figure 5: Scavenging Effects of Different Extract of *Emilia praetermissa* Leaves on Hydroxyl radical. The results are means ± SD (n=3).

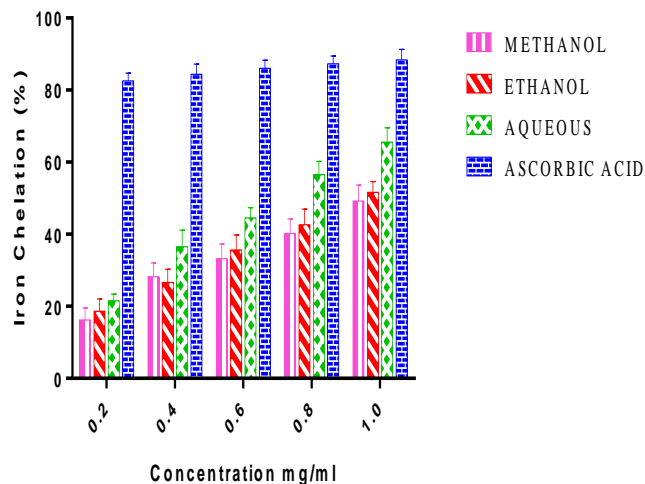


Figure 6: Iron Chelation Ability of Different Extract of *Emilia praetermissa* leaves. The results are means ± SD (n=3).

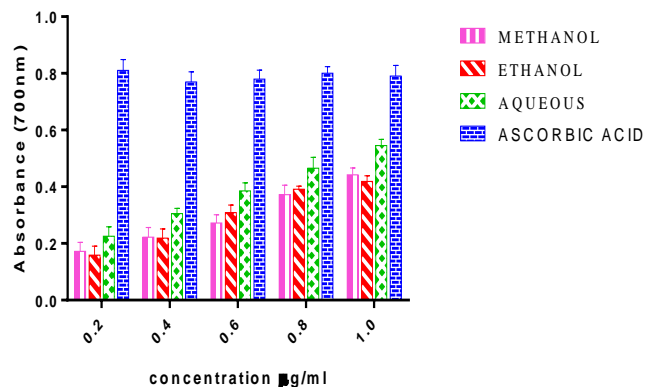


Figure 7: Ferric Reducing Ability of Different Extract of *Emilia praetermissa* leaves. The results are means ± SD (n=3).

IV. CONCLUSION

The results of this study established that the Aqueous extract of *Emilia praetermissa* leaves possess potent antioxidant

components in form of phytochemicals which free radical scavenging potential. *Emilia praetermissa* leaves could be used in the management of oxidative stress related diseases.

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