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Evaluation of antioxidant activity of stem and flower extracts of Ageratum conyzoides

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ABSTRACT

The study aimed to evaluate and compare antioxidant activity couple with a phytochemical screening of different extracts from stems and flowers of Ageratum conyzoides. N-hexane, ethyl acetate, and ethanolic extracts were subjected to different phytochemical screening and antioxidant activity. The flavonoids and phenolic contents were determined using spectrophotometry method. The three extracts showed evidence for the presence of secondary metabolites such as flavonoids, steroids, terpenoids, and glycosides. Saponins were only present in ethanol extract. Phenol was present in both ethanol, ethyl acetate, and n-hexane extracts. The concentration of flavonoids ranges from 145.33 ± 0.665 to 711.00 ± 2.024 µg/g expressed as Quercetin equivalents. Total phenolic content expressed as Gallic acid equivalents range from 19.26 ± 0.305 to 150.33 ± 1.020 µg/g of extract. The DPPH inhibition was also determined using spectrophotometry method and it was observed between 34.65 to 84.90%. The results obtained in this study show that the stems and flowers of Ageratum conyzoides are rich in flavonoids and phenols. Thus, the plant possesses scavenging property.

Keywords: *Ageratum conyzoides, Phytochemicals, Phenolic contents, Flavonoids, Antioxidants.*

1. INTRODUCTION

Ageratum conyzoides is an annual branching herb which grows to approximately 1 m in height. The stems and leaves are covered with fine white hair; the leaves are ovate and up to 7.5 cm long. It has a shallow tap root system. The flowers are purple to white, less than 6 mm across and arranged in close terminal inflorescences. The fruits are achene and are easily dispersed while the seeds are often lost within 12 months. The seeds germinate at a room temperature of 20-25 ºC. It prefers a moist, well-drained soil but may tolerate dry conditions [1, 2]. The plants grow commonly in the proximity of habitation, thrives in any garden soil and are very common in waste places and on ruined sites. It has a peculiar odor likened in Australia to that of male goat and hence its name 'goatweed or billy goat weed'. The species has great morphological variation and appears highly adaptable to different ecological conditions [3]. As many plants produce a significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen radicals, they represent a potential source of new compounds with antioxidant activity. Antioxidants help organisms deal with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability [4]. It is possible to reduce the risks of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants [5]. Although different plants have been analyzed for their medicinal properties, there is always a need for isolation of novel chemotherapy from natural sources with different modification to prevent or cure oxidative stress which are the major causes of diseases such as diabetes, heart diseases, cancer, tumors etc.

The plant *Ageratum conyzoides* is used for wound dressing, curing various skin diseases, ophthalmic, colic, ulcers treatment, as purgative and febrifuge. Other folk remedies include anti-itch, sleeping sickness, and mouthwash for a toothache, antitussive, tonic and killing lice [6, 7]. It also exhibits marked hepatoprotective action, which has been connected to the plants with antioxidant properties. For these reasons, several leading pharmaceutical companies have started entering into the field of medicinal plant research for the production of drugs [8]. This present study investigates the phytochemicals and antioxidant properties of n-hexane, ethyl acetate and ethanolic extracts from stems and flowers of *Ageratum conyzoides*.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh plants of *Ageratum conyzoides* were collected within the premises of University of Ilorin, Ilorin, Nigeria. The plants were authenticated by Dr. Adeyemi S.B of the Department of Plant Biology, University of Ilorin and samples deposited at the herbarium unit with the herbarium number UIH001/810 issued.

2.2 Extraction

Stems and flowers of the plant were air dried separately at ambient temperature (25 ^{o}C) and pulverized after drying. Thereafter, 400 g of the pulverized sample was soaked with n-hexane in a stoppered glass container for five days with frequent agitation [9]. The crude extract was decanted and filtered through cotton plug funnel and Whatman no.1 filter paper. The filtrate from the n-hexane extraction was concentrated under vacuum using rotary evaporator. Subsequent extraction with ethyl acetate and ethanol was done following the same procedure respectively.

2.3 Phytochemical Screening

2.3.1 Qualitative analysis of phytochemicals

Phytochemical constituents of the crude extracts of *Ageratum conyzoides* were screened qualitatively using standard procedures [10, 11, 12, 13] to identify the presence of various primary and secondary metabolites in the extracts such as alkaloids, tannins, anthraquinones, saponins, flavonoids, terpenoids, glycosides, steroids, and phenols.

2.3.2 Total phenolic contents

The total phenolic content in the plant extracts was determined using spectrophotometric method [14]. 1, 5 and 10 mg/ml of a methanolic solution of the extract was prepared and used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic extract with 2.5 ml of 10 % Folin-Ciocalteu's reagent and 2.5 ml 7.5 % $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10 % Folin-Ciocalteu's reagent and 2.5 ml of 7.5 % of $NaHCO_3$. The mixture thereafter undergoes incubation in a thermostated water bath in dark cupboard at 45 ^{o}C for 45 min. The absorbance was then determined at a wavelength of 765 nm using spectrophotometer (Deckmann GU750). The mixture was prepared in triplicate for each analysis and the mean value of absorbance was obtained. A solution of Gallic acid which serves as a standard was prepared in methanol to achieve the concentration of 0.5 mg/ml and dilution was made to obtain concentrations of 25, 12.5, 6.25, 3.13, 1.56 and 0.78 µg/ml, and the calibration line was constructed. Based on the measurement of the absorbance, the concentration of phenolics was determined (µg/ml) from the calibration line and the total phenolic content in the extracts was expressed in terms of Gallic acid equivalent (mg of GA/g of extract).

2.3.3 Flavonoid contents

The total flavonoids in the plant extracts were determined using spectrophotometric method [15]. Methanol was used in preparing extracts concentrations of 1, 5 and 10 mg/ml which was used in the analysis. 1 ml of each solution of the extracts was added to 1 ml of 2 % $AlCl_3$ solution prepared in methanol. The samples undergo incubation for an hour at room temperature and the absorbance was then read using spectrophotometer at a wavelength of 415 nm (Deckmann GU750). The samples were prepared in triplicate for each analysis to obtain the mean value of absorbance. The quercetin equivalent and a calibration curve were constructed by preparing quercetin solutions at concentrations varying from 10 - 100 µg/ml in methanol. Based on the measured absorbance, the concentration of flavonoids was determined (µg/ml) on the calibration line and the flavonoid contents in the extracts were expressed in terms of Quercetin equivalent (mg of Q/g of extract).

2.4 Evaluation of Antioxidant activity

The ability of the plant extract to scavenge DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radicals was assessed by the method of [16, 17]. The stock solution of extracts was prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.99 and 0.97 µg/ml. The prepared solution (1 ml) was mixed with 1 ml of a methanolic solution of DPPH of concentration 1 mg/ml. After 30 min of incubation in dark at room temperature, the absorbance was then recorded at 517 nm with Spectrophotometer. A control sample containing 1ml of methanol and 1 ml of methanolic DPPH solution was set alongside. Percentage inhibition was calculated using the equation below, whilst IC_{50} (inhibitory concentration at 50 %) was estimated from the percentage inhibition versus log concentration plot, using a non-linear regression algorithm.

$$\%\ inhibition = \left(\frac{A\ of\ control - A\ of\ sample}{A\ of\ control}\right) \times 100$$

3. RESULTS AND DISCUSSION

Phytochemical constituents of the crude extracts are shown in Table 1. It revealed the presence of alkaloids, glycosides, steroids, terpenoids, phenols, and flavonoids in all the crude extracts of both stems and flowers of *Ageratum conyzoides*. Saponins were only observed in the ethanol and ethyl acetate extracts of the stems, but Saponins was found only in crude ethanol extract of the flowers. Most of the metabolites were recorded in the ethanol extracts except for saponins. Ethanol being a polar solvent and close in polarity to water lead credence to efficiency and usefulness of the aqueous extracts of this plant as remedies for several diseases in folk medicine. These phytochemicals present in plants are connected with the therapeutic efficiencies and efficacies of medicinal plants [18]. The presence of phenols and saponins in these stems or flowers could be used to justify the use of *Ageratum conyzoides* in treating wounds, prevention of blood loss, etc. [19]. The presence of phenol in the investigated crude extracts indicates that *Ageratum conyzoides* can be used as an antioxidant agent. This is because phenol and phenolic compounds have been extensively used in

disinfection [13]. Flavonoids have the ability to scavenge hydroxyl radicals, super oxide anions and lipid peroxy radicals [20]. Phytochemicals act in numerous ways to assist the body in combating diseases and health problems. They combine with some biomolecules to neutralize activity and scavenging free radicals before they can cause damage within the body [21].

Table 1: Phytochemical constituents from the stems and flowers extracts of *Ageratum conyzoides*

Phytochemical constituents	**Test**	**Stems**			**Flowers**		
		n-hexane	Ethyl acetate	Ethanol	n-hexane	Ethyl acetate	Ethanol
Alkaloids	Dragendroff	+	++	+++	+	+	++
	Wagner test	+	++	+++	+	+	++
Saponins	Frothy	-	+	+	-	-	+
Flavonoids	Shinoda	+	+	+	+	+	+
Steroids	Liberman-burchard	+	+	+	+	+	+
Terpenoids	Noller's test	+	+	+	+	+	+
Cardiac glycosides	Keller killiani	+	+	+	+	+	+
Phenols	Ferric chloride	+	+	+	+	+	+

Key: +++ strongly present, ++ moderately present, + weakly present, - absent.

The Calibration curve of standard gallic acid for the determination of total phenolic content in the stem and flowers extracts of *Ageratum conyzoides* is shown in Figure 1. The total phenolic contents of the *Ageratum conyzoides* extracts using the Folin Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: $y = 0.005x$, $R^2 = 0.990$)

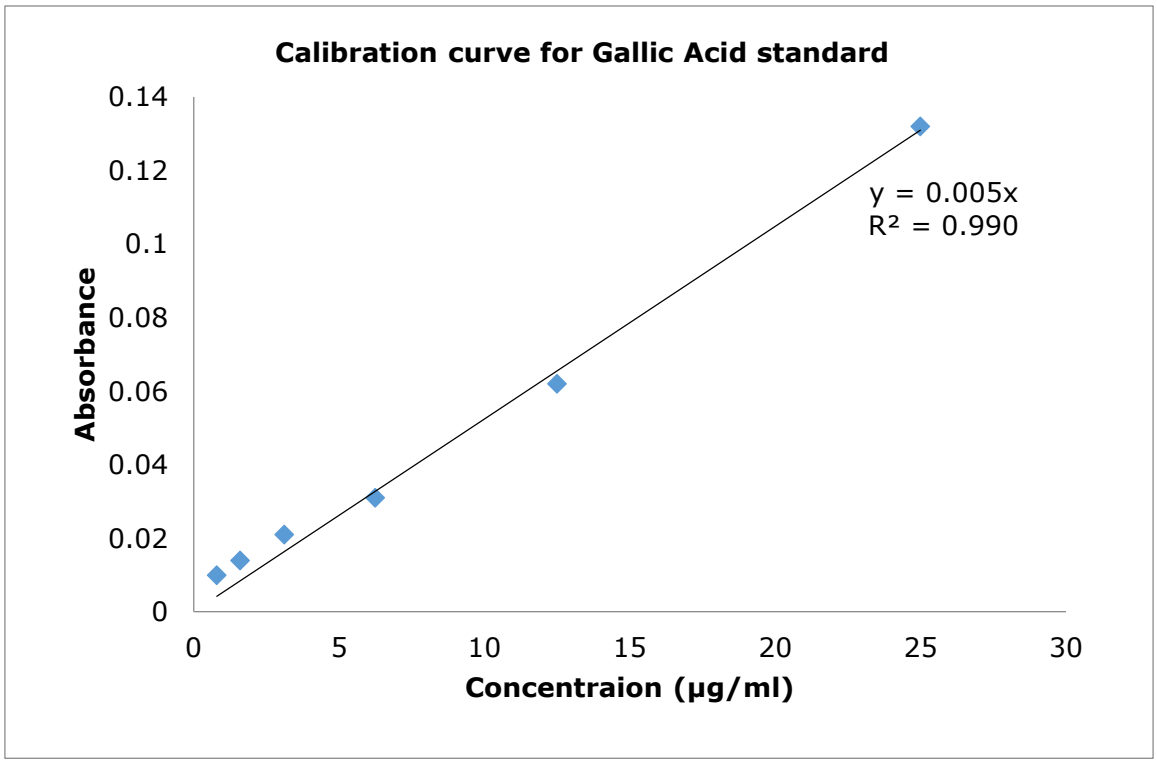

Figure 1: Calibration curve of standard gallic acid for the determination of total phenolic content in the stem and flowers extracts of *Ageratum conyzoides.*

Estimated phenolic contents in the extracts of stems and flowers of *Ageratum conyzoides* at various concentrations are presented in Figure 2. The total phenolic contents in the examined crude extracts range from 19.26 ± 0.305 to 150.33 ± 1.020 µg GA/g. Phenolic contents of the crude n-hexane, ethyl acetate and ethanolic stem and flower extracts of *Ageratum conyzoides* calculated using y = 0.005x ranged from 25.27 ± 3.202 to 150.33 ± 1.020 µg/g and 19.26 ± 0.305 to 107.33±2.500 µg/g respectively at different concentration. Ethanolic extract of the stem at a concentration of 10 mg/ml recorded the highest phenolic content with 150.33 ±

1.020 µg/g followed by an ethanolic extract of the flowers with a 107.33 ± 2.50 µg/g at the same concentration. This followed the same trend in the concentration-dependent manner for crude ethyl acetate and n-hexane extracts from the stems and flowers extract of *Ageratum conyzoides.* N-hexane extract from the stem and flowers of *Ageratum conyzoides* recorded the lowest phenolic content at 25.27 ± 3.202 µg/g and 19.26 ± 0.305 µg/g respectively.

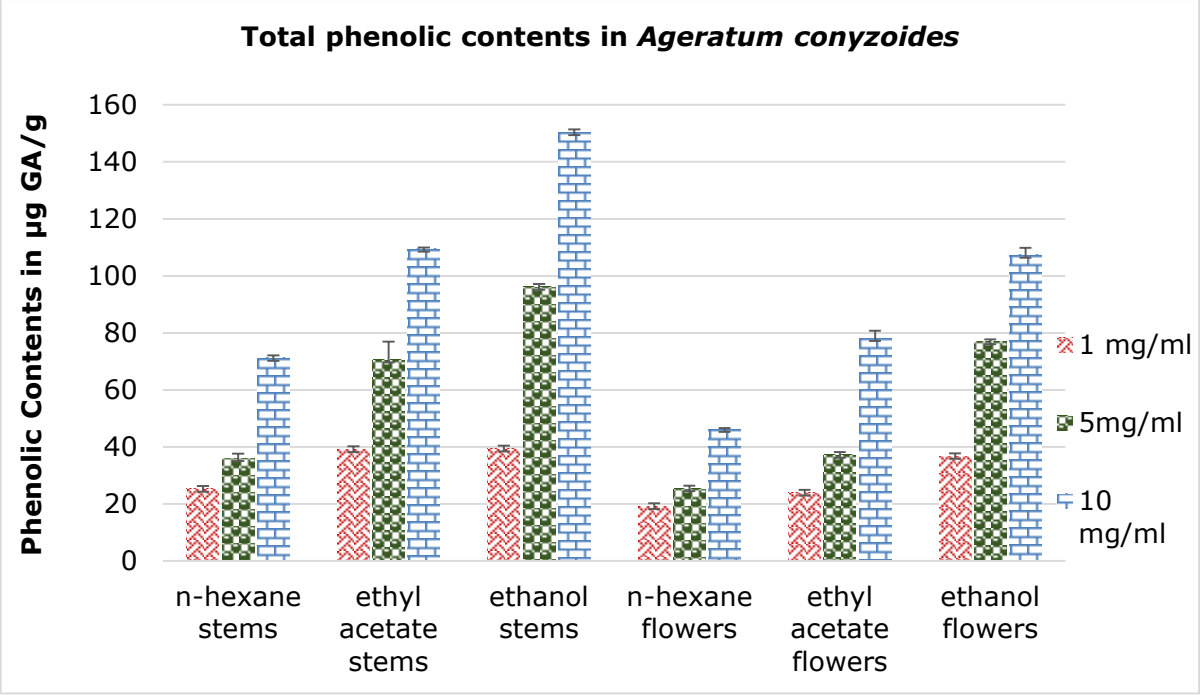

Figure 2: Estimated phenolic contents in the stems and flowers extract of *Ageratum conyzoides* at 1, 5 and 10 mg/ml

The concentration of flavonoids was expressed in terms of quercetin equivalent and a calibration curve was constructed by preparing quercetin solutions at concentrations of 10 to 100 µg/ml in methanol (the standard curve equation: $y = 0.003x$, $R^2 = 0.988$), µg of QE/g of extract (figure: 3).

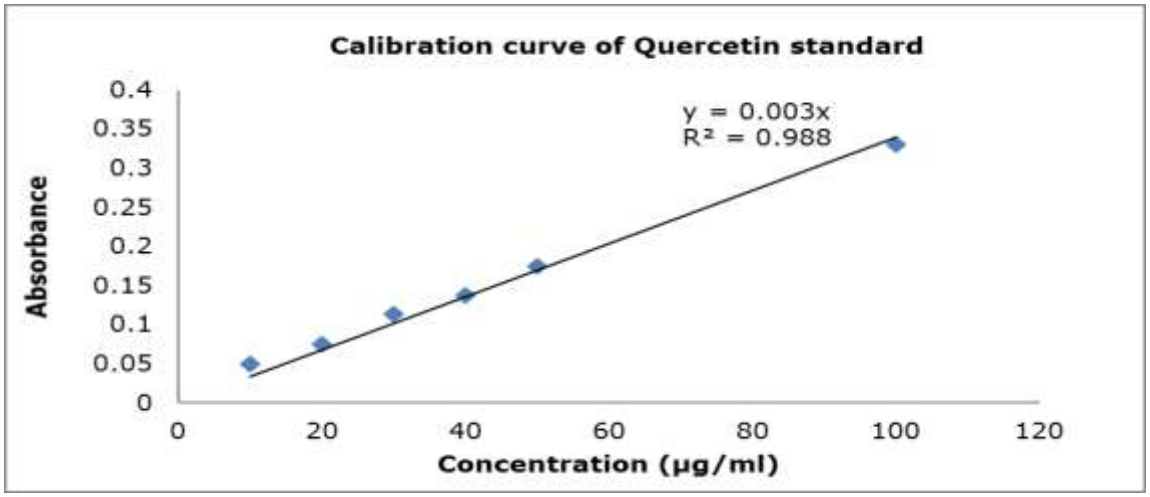

Figure 3: Standard curve of quercetin for the estimation of flavonoid concentrations in *Ageratum conyzoides* extracts

Estimated flavonoid concentrations in the stems and flowers of *Ageratum conyzoides* at various concentrations are presented in Figure 4. The concentration of flavonoids in stems and flowers extract from *Ageratum conyzoides* ranged from 209.00 ± 2.903 to 711.00 ± 2.024 µg/ml. Generally, ethanol extracts recorded significantly higher concentration of flavonoids in both stem and flowers. Ethanolic extract of the stems at a concentration of 10.00 mg/ml recorded the highest flavonoids concentration with 711.00 ± 2.024 µg/g of Quercetin/g followed by the ethanolic extract of the flowers with 597.11 ± 1.168 µg/g of Quercetin/g at the same concentration respectively. This followed the same trend in concentration-dependent manner for crude ethyl acetate and n-hexane extracts from stems and flowers of *Ageratum conyzoides.* The crude n-hexane extract from the stems and flowers of the plant recorded the lowest concentration of flavonoids with 209.00 ± 2.903 µg/g of Quercetin/g and 145.33 ± 0.665 µg/g of Quercetin/g respectively as shown in figure 4 above. The quantities of the flavonoids extracted increases with the polarity of solvent used for the extraction. This result is in agreement with the observation of others in extraction techniques of flavonoids [22].

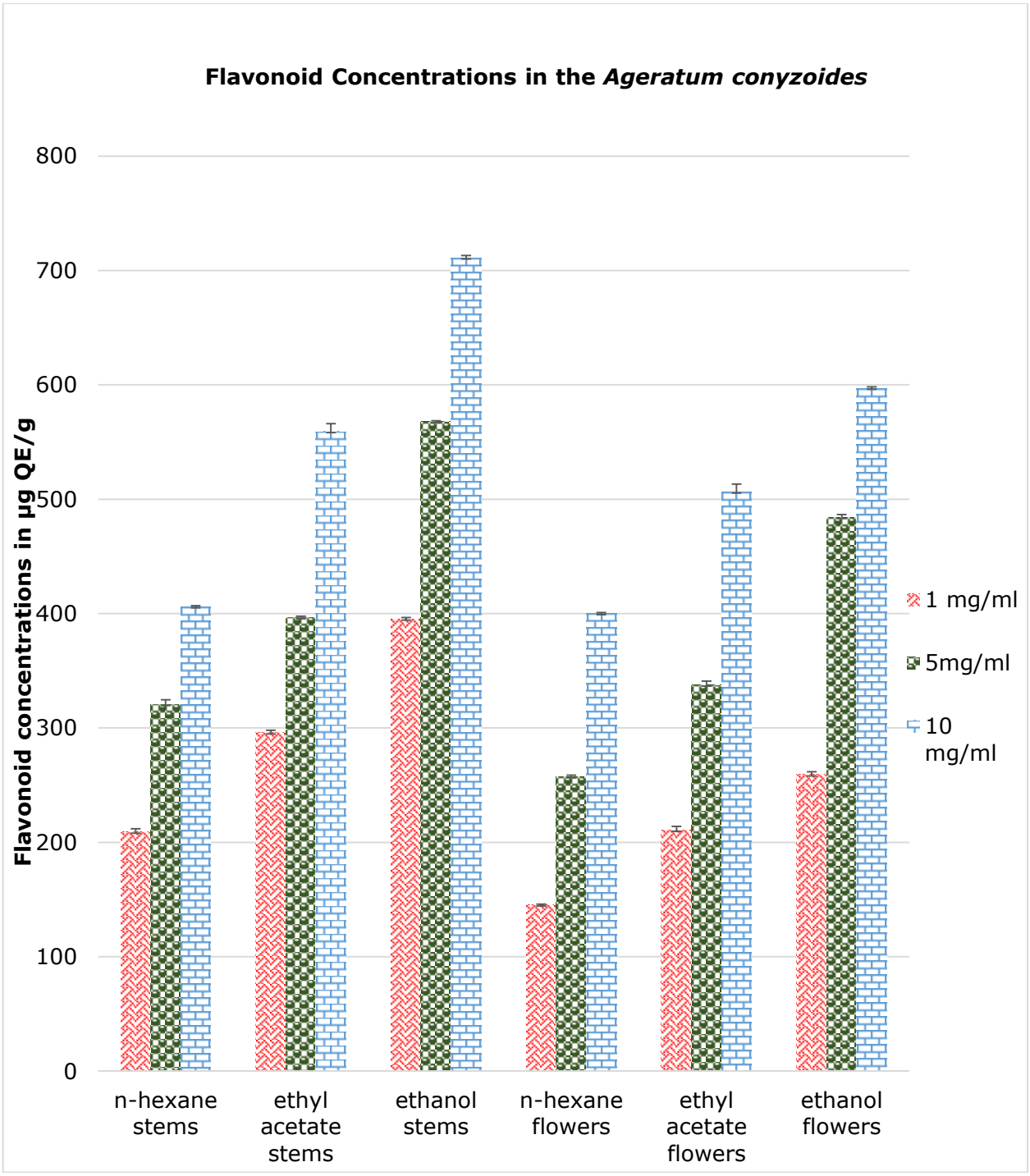

Figure 4: Estimated flavonoid concentrations in the stems and flowers of *Ageratum conyzoides* at 1, 5 and 10 mg/ml of each extract

The DPPH scavenging activity of extracts of stems and flowers of *Ageratum conyzoides* are presented in Tables 5 and 6 respectively. The largest capacity to neutralize DPPH radicals was found in ethanolic stems and flowers extract of *Ageratum conyzoides* which neutralized 50% of free radicals at the concentrations of 7.8µg/ml and 11.71µg/ml respectively. A moderate activity was found for ethyl acetate stem and flower at the concentrations of 27.43µg/ml and 46.87µg/ml respectively. In comparison to IC_{50} values of Ascorbic acid which was found to be 5.66µg/ml, enough to scavenge DPPH radical. This is considered to be chemotherapeutically significant (IC_{50} <10 µg/ml) with a potency that could be used as a reference [25]. Phenol and flavonoids are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, the phenolic content of these extracts may contribute directly to their antioxidant action [23]. For instance, the extracts that possessed the highest concentration of phenols in this study possessed highest antioxidant activity. This finding is consistent with the report of others [24]. The radical scavenging activity of the extracts could also be linked to the high concentration of flavonoids in *Ageratum conyzoides*. Several studies have reported that presence of flavonoids in herbs significantly contributed to their antioxidant properties and healing effect because of their ability to scavenge practically all known reactive species [26]. These data are in agreement with other studies reporting the concentration of flavonoid in different parts of the plant [27].

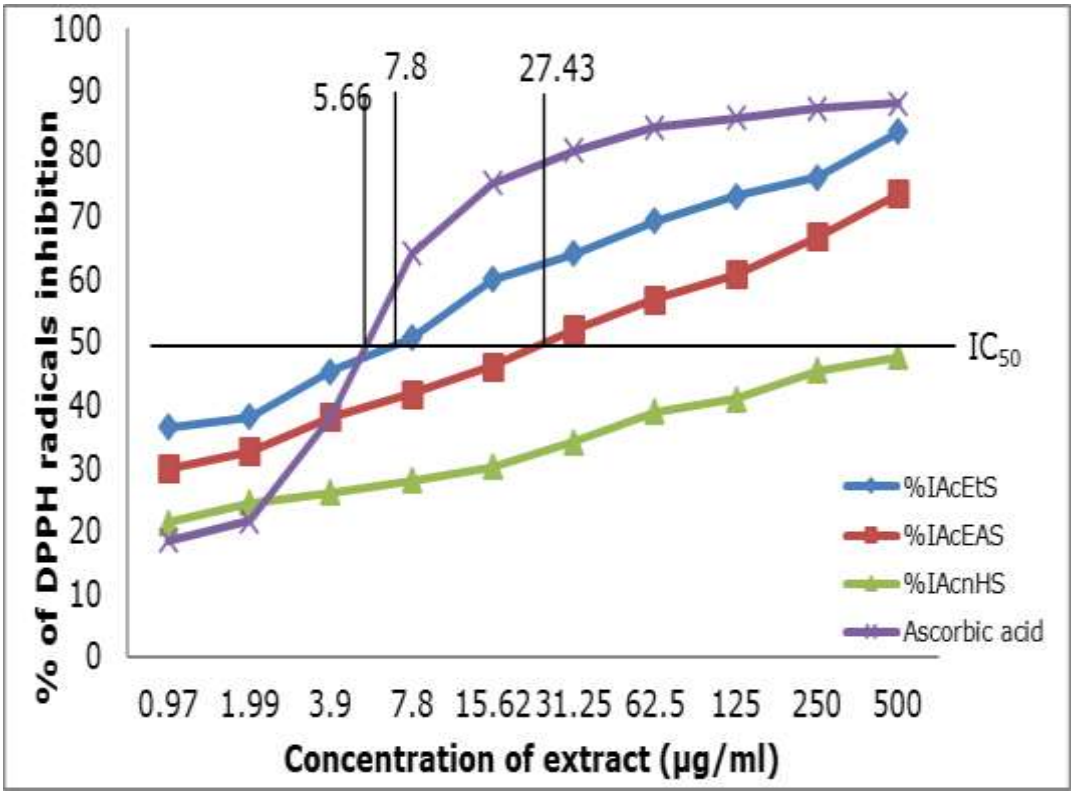

Figure 5: Antioxidant (DPPH scavenging) activity of investigated stem extracts presented as a percentage of DPPH radical's inhibition and IC_{50} values (µg/ml)

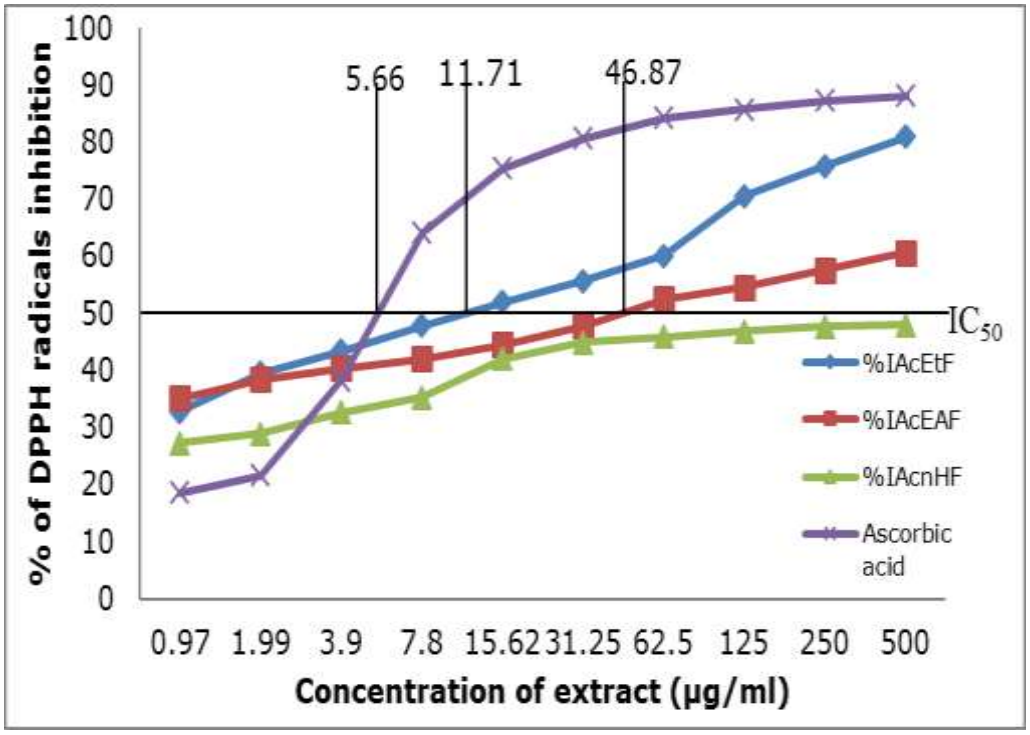

Figure 6: Antioxidant (DPPH scavenging) activity of investigated flower extracts presented as a percentage of DPPH radicals inhibition and IC_{50} values (µg/ml)

KEY: **%I –** %inhibition, **Ac –** *Ageratum conyzoides*, **Et –** Ethanol, **EA –** Ethyl acetate, **nH –** n-hexane, **S –** stem, **F –** flower.

4. CONCLUSION

The present study revealed that stems and flowers of *Ageratum conyzoides* contain an abundance of flavonoids and phenols and that the efficiency of extraction of phytochemicals in the stem and flower of the plant increases with the polarity of the solvents. The high concentration of phenol and flavonoid in the stem and flower extracts might be responsible for the antioxidant activity exhibited

by the plant. The results of this study indicated that stem and flowers of *Ageratum conyzoides* species could be seen as a potential candidate for the development of an alternative therapy to combat free radicals-induced oxidative stress and oxidative stress-associated diseases.

Further isolation and characterization of bioactive compounds in this plant as well as *in vivo* studies could be useful in developing a new therapeutic agent from this plant.

5. ACKNOWLEDGEMENT

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