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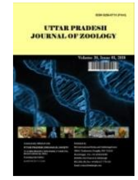

VERMICOMPOST SUPPLEMENTATION ON ANTIOXIDANT POTENTIAL OF *Trigonella foenum-graecum Linn*

M. PARVEEN[1*] AND V. ANURADHA[1]
[1]Post Graduate and Research Department of Zoology, Justice Basheer Ahmed Sayeed College for Women, Chennai, India.

AUTHORS' CONTRIBUTIONS
This work was carried out in collaboration between both authors. Author MP designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Both authors managed the literature searches, read and approved the final manuscript.

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Original Research Article

ABSTRACT

Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing free radical scavenging activity. Vermicompost is an organic materials broken down by the interactions between microorganisms and earthworms in a mesophilic process to produce fully stabilized and organic soil amendments with low carbon to nitrogen (C:N) ratios Vermicompost is rich in NPK micronutrients, and beneficial soil microbes, so it is garnering attention as a greener replacement for chemical fertilizers to maintain and further improve soil quality. In the present study effects of vermicompost supplementation on antioxidant activities of *Trigonella foenum-graecum* was investigated. Anecic earthworm *Lampito mauritii,* Vermicompost, Farmyard manure and seeds of *Trigonella foemum-graecum*. L was inoculated in to soil at different treatments. Seeds of *Trigonella foemum-graecum* treated with 0.1% Mercuric chloride for 2 minutes and washed with running water to remove contamination of seed coat, prior to germination studies. The experimental setup was designed and it was classified in to six groups. *Trigonella foenum – graecum* was plugged out from the different treatment experimental setup at after 15 days. Antioxidant activity was determined by DPPH free radical scavenging assay. DPPH scavenging activity of ethylacetate, ethanol and aqueous extract of *Trigonella foenum-graecum* with different treatments was studied. Out of which ethanol extracts of *Trigonella foenum-graecum* exhibits maximum level of DPPH free radical scavenging activity and group IV possessed the good activity when compared to other groups due to presence of vermicompost and *Lampito maurtii.* The extract from *Trigonella foenum- graecum* treated with vermicompost and *Lampito marutii* showed better antioxidant activity when compared to other groups. The present study suggests that *Trigonella foenum-graecum* could be used as natural antioxidants and nutraceuticals to enhance the health benefits.

Keywords: *Trigonella foenum-graecum; Lampito mauritii;* vermicompost; farmyard manure; DPPH activity.

1. INTRODUCTION

Vermicompost is an organic waste produced by earthworms namely *Lampito mauritii*, *Eudrilus eugeniae* and *Perionyx excavatus* from interaction with microorganisms in a mesophilic process, to produce fully stabilized and organic soil enhancements that are rich with essential plant

**Corresponding author: Email: manojdhanrajk@gmail.com;*

nutrients. Vermicomposting aids in diverting organic wastes from landfills and serves as a quick and cost-effective method of composting. Various studies have reported that enhancement of soil using vermicompost elevate soil quality, resulting in improved soil structure, increased microbial activity, and enhanced plant available nutrients, which in turn improves plant production [1]. The benefits that medicinal plants receive from vermin composting depend on the plant's ability to extract from the fertilizing substrate and the substances needed for growth and development [2]. A limited number of reports were found in the published literature on the effect of vermicompost supplementation on antioxidant activities of *Trigonella foenum-graecum Linn*.

Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules viz., nucleic acids, proteins, lipids and DNA and can initiate different degenerative diseases like diabetes, inflammation, atherosclerosis, ischaemic heart disease and ageing process [3,4]. The mechanisms of protective actions of antioxidants against ROS toxicity include prevention of the formation of ROS, interruption of ROS attack, scavenging of the reactive metabolites or their conversion to stable molecules or molecules of lower reactivity [5]. In this regard, antioxidant complements, or foods encompassing antioxidants, could assist the body's defense system and contribute to the reduction or neutralization of oxidative damages.

Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects, while relatively plant-based medicines confer fewer side effects than the synthetic drug in some instances [6]. Plants are well known to produce a diverse array of secondary metabolites to engage with the world around them. The major classes of secondary metabolites are alkaloids, terpenes and phenols. Many medicinal plants, vegetables and fruits have antioxidant components, especially phenolic compounds, which when consumed, have been confirmed to prevent the destructive and degenerative effects caused by oxidative stress [7]. Numerous naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective and they are known to inhibit lipid peroxidation (by inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions [8] The DPPH method was evidently introduced nearly 50 years ago by Blois [9] and it is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant capacity. The free radical scavenging activity of plant extract was studied by its ability to reduce the DPPH, a stable free radical and any molecule that can donate an electron or hydrogen to DPPH. It can react with it and thereby bleach the DPPH absorption [10]. Hence the present study was undertaken to elucidate the vermicompost supplementation improves the stability of soil and its antioxidant potential on *Trigonella foenum-graecum Linn.*

2. MATERIALS AND METHODS

2.1 Collection of Samples

The Anecic earthworm *Lampito mauritii* was collected from vermiculture farm, Chennai. The vermicompost and farmyard manure were collected from Priya Horticulture, Chennai. *Trigonella foemum-graecum*. L (fenugreek) seed collected from local market, Chennai.

2.2 Preparation of Samples

Garden soil was collected from Chennai and sieved to remove the debris. Anecic earthworm *Lampito mauritii,* Vermicompost, Farmyard manure and seeds of *Trigonella foemum-graecum* L. were inoculated in to soil at different treatments. Seeds of *Trigonella foemum-graecum* treated with 0.1% Mercuric chloride for 2 minutes and washed with running water to remove contamination of seed coat, prior to germination studies.

2.3 Preparation of *Trigonella foenum-graecum* Extract

The leaves and stem of *Trigonella foenum-graecum* were collected from above experiments and cleaned, shade dried and coarsely powdered. Successive solvent extraction was done by cold percolation method [11] by soaking in ethyl acetate and ethanol successively in an aspirator bottle for 48 h. Simultaneously, Aqueous extracts of leaves and stem of *Trigonella foenum-graecum* were also prepared. After 48 h, the extracts were filtered by Whatman Filter paper No.1. The solvent was removed by distillation using Rotary Evaporator and the extracts were concentrated and dried in Freeze Dryer.

2.4 Antioxidant Effect of *Trigonella foenum graecum* Grown in Different Treatments by Using DPPH Free Radical Scavenging Assay

2.4.1 Procedure

The ability of the samples to annihilate the DPPH radical (1,1-diphenil-2-picrylhydrazyl) was investigated by the method described by Blois [9]

Table 1. Experimental set up

S. no	Groups
1	**Group I** – Soil
2	**Group II** - Soil + *Lampito mauriti*,
3	**Group III** - Soil + Vermicompost
4	**Group IV** - Soil + Vermicompost + *Lampito mauriti*,
5	**Group V** - Soil + Farmyard manure
6	**Group VI** - Soil + Farmyard manure + *Lampito mauriti*

Stock solution of compound was prepared to the concentration of 10 mg/ml. Different concentration of the extract (200, 400, 600, 800, 1000 µg) of sample were added, at an equal volume to methanolic solution of DPPH (0.1 mM). The reaction mixture is incubated for 30min at room temperature; the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid was used as standard control. The annihilation activity of free radicals was calculated in percentage inhibition according to the following formula

$$\text{\% of Inhibition} = \frac{\text{A of control} - \text{A of Test}}{\text{A of control}} \times 100$$

3. RESULTS AND DISCUSSION

The present study elucidated the antioxidant activity of *Trigonella foenum-graecum* with different treatments. DPPH scavenging activity of ethylacetate, ethanol and aqueous extract of *Trigonella foenum-graecum* with different treatments was studied. DPPH scavenging activity of *Tf*EaE showed the inhibition activity in all the treatment groups. DPPH radical scavenging activity is one of the most widely used method for screening the antioxidant activity of medicinal plants. Antioxidants on interaction with DPPH transfer electrons or hydrogen atoms to DPPH, thus neutralizing free radical character. The colour of the reaction mixture changes from purple to yellow and its absorbance at wavelength 517 nm [12]. In ethyl acetate extract of *Trigonella foenum-graecum,* Group I and V showed the maximum inhibition activity at maximum concentration (1000 µg) and it was found to be 32.23% followed by other groups. Positive control showed 91% of inhibition at 1000 µg. Ethyl acetate extract of *Trigonella foenum- graecum* significantly inhibited the DPPH free radical scavenging activity. Percentage of inhibition was dose dependent manner (P<0.05) (Table 2 and Fig. 1).

Similarly, maximum inhibitory percentage of inhibition was observed in ethanol extract of *Tf*EE (Group IV) and it was found to be 74.06% at 1000 µg. Group VII and II also showed a maximum level inhibition activity which represented the antioxidant activity. Moderate activity was observed at Group V and it was found to be 52.28%. Ethanol extract of *Trigonella foenum-graecum* significantly inhibited the DPPH free radical scavenging activity (P<0.05). Aqueous extracts of *Trigonella foenum-graecum* also showed the DPPH free radical scavenging activity and the maximum level of inhibition was found at 1000µg and the value was 39.51% at group III (Tables 3 - 4 and Figs. 2-3). Other treatment groups also showed the DPPH free radical scavenging activity. Aqueous extract of *Trigonella foenum-graecum* significantly inhibited the DPPH free radical scavenging activity. Percentage of inhibition was dose dependent manner (P<0.05).

Likewsie, Reddy and Grace [13] also evaluated the antioxidant activity of methanolic extracts of selected mangrove plants namely *Aegiceras corniculatum, Excoecaria agallocha* and *Lumnitzera racemosa*. Antioxidants are important substances that play a crucial role in delaying, intercepting, and preventing oxidative reactions catalyzed by free radicals and thus providing protection to humans [14]. Due to this special ability there is an increased use of antioxidants for the balance of reactive oxygen species. DPPH has been used extensively as a free radical to evaluate reducing substances and is a useful reagent for investigating the free radical scavenging activities of compounds [15,16]. Our results show high DPPH scavenging activity. Many researchers have reported positive correlation between free radical scavenging activity and total phenolic and flavonoid contents, which also matches with our findings. Medicinal plant tissues are commonly rich in phenolic compounds such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins.

Although the DPPH radical scavenging abilities of the extracts were significantly lower than that of control, it was evident that the extracts showed proton-donating ability and this could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. The effect of antioxidant on DPPH is believed to be due to their hydrogen-donating ability. The antioxidative activities observed can be attributed to either the different mechanisms exhibited by different polyphenolic compounds that is, tocopherols, flavonoids and other organic acids and to

Table 2. DPPH scavenging activity of ethylacetate extract of *Trigonella foenum-graecum* with different treatments

Conc (µg)	Percentage inhibition							
	Groups							Positive control
	I	II	III	IV	V	VI	VII	
200	8.21 ± 0.12	7.21 ± 0.11	5.43 ± 0.09	8.54 ± 0.06	5.69 ± 0.03	5.43 ± 0.03	6.68 ± 0.04	39.77 ± 0.24
400	24.69 ± 0.21	12.31 ± 0.10	7.21 ± 0.01	13.37 ± 0.11	9.40 ± 0.08	16.15 ± 0.08	7.21 ± 0.03	52.88 ± 0.34
600	27.73 ± 0.23	21.31 ± 0.20	10.66 ± 0.08	19.06 ± 0.12	25.22 ± 0.13	21.11 ± 0.12	9.20 ± 0.04	58.77 ± 0.39
800	29.72 ± 0.26	25.88 ± 0.19	16.15 ± 0.10	22.57 ± 0.18	29.05 ± 0.18	25.35 ± 0.21	12.05 ± 0.08	77.10 ± 0.42
1000	32.23 ± 0.28	27.86 ± 0.23	18.93 ± 0.12	30.38 ± 0.16	32.23 ± 0.19	31.70 ± 0.24	14.89 ± 0.10	91.00 ± 0.52

Table 3. DPPH scavenging activity of ethanol ethanol extract of *Trigonella foenum-graecum* with different treatments

Conc (µg)	Percentage inhibition							
	Groups							Positive control
	I	II	III	IV	V	VI	VII	
200	25.15 ± 0.21	14.30 ± 0.07	14.69 ± 0.09	17.14 ± 0.09	15.09 ± 0.12	14.16 ± 0.07	14.23 ± 0.07	39.77 ± 0.18
400	29.05 ± 0.22	25.68 ± 0.19	25.41 ± 0.21	31.97 ± 0.20	28.39 ± 0.20	25.61 ± 0.9	29.05 ± 0.18	52.88 ± 0.25
600	34.35 ± 0.27	30.44 ± 0.23	36.66 ± 0.28	46.00 ± 0.22	39.05 ± 0.24	35.74 ± 0.23	37.00 ± 0.24	58.77 ± 0.27
800	46.26 ± 0.31	46.26 ± 0.32	52.42 ± 0.31	59.30 ± 0.28	41.50 ± 0.31	46.19 ± 0.28	52.42 ± 0.27	77.10 ± 038
1000	59.36 ± 0.44	58.77 ± 0.40	61.48 ± 0.42	74.06 ± 0.41	52.28 ± 0.24	59.36 ± 0.32	63.00 ± 0.30	91.00 ± 0.44

Table 4. DPPH scavenging activity of aqueous extract of *Trigonella foenum-graecum* with different treatments

Conc(µg)	Percentage inhibition							
	Groups							Positive control
	I	II	III	IV	V	VI	VII	
200	1.26 ± 0.001	7.88 ± 0.04	9.20 ± 0.05	9.20 ± 0.05	8.41 ± 0.05	12.24 ± 0.06	14.23 ± 0.08	39.77 ± 0.18
400	10.52 ± 0.04	15.09 ± 0.08	21.11 ± 0.12	21.11 ± 0.12	14.36 ± 0.09	14.23 ± 0.07	18.13 ± 0.09	52.88 ± 0.25
600	21.77 ± 0.13	23.10 ± 0.11	29.05 ± 0.15	28.92 ± 0.18	25.68 ± 0.12	21.64 ± 0.11	21.51 ± 0.18	58.77 ± 0.29
800	28.59 ± 0.14	32.16 ± 0.15	34.35 ± 0.16	31.90 ± 0.16	28.86 ± 0.17	31.97 ± 0.19	30.31 ± 0.16	77.10 ± 0.36
1000	32.16 ± 0.16	35.61 ± 0.16	39.51 ± 0.18	35.47 ± 0.18	34.22 ± 0.18	33.36 ± 0.18	35.01 ± 0.18	91.00 ± 0.46

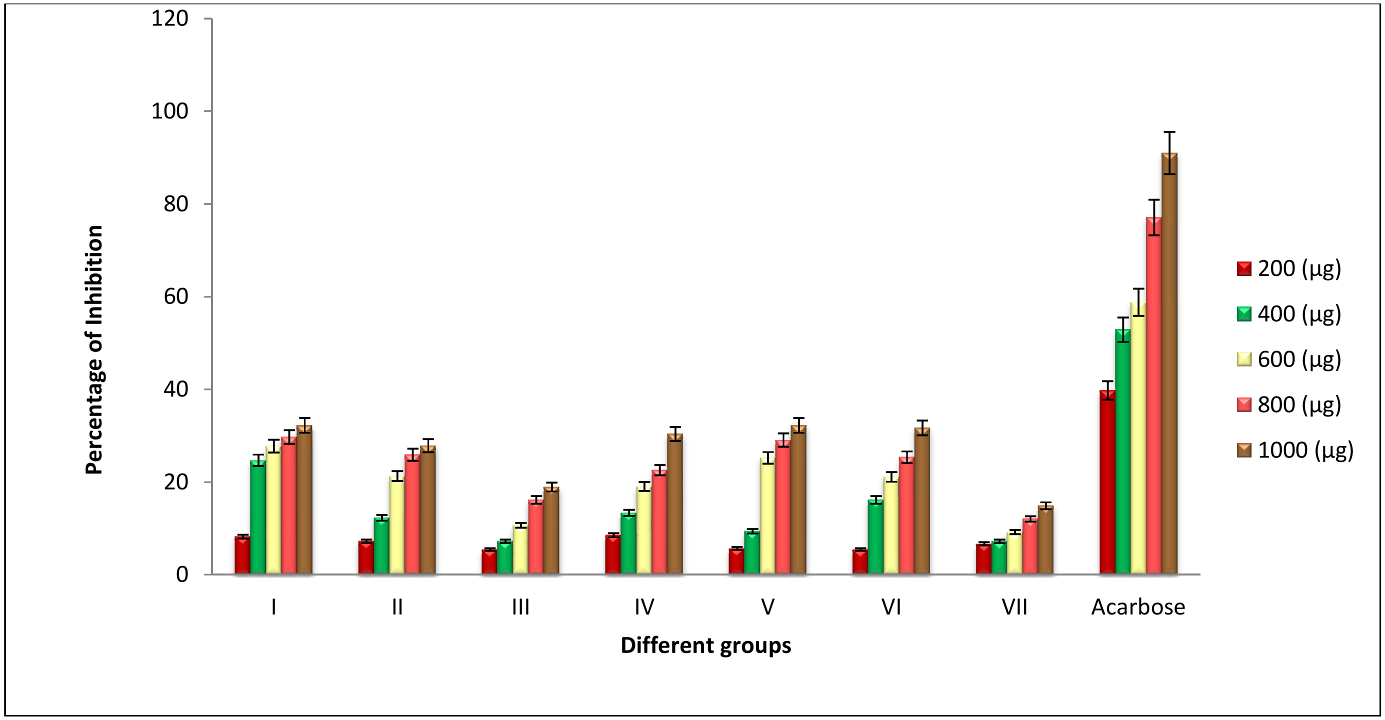

Fig. 1. DPPH scavenging activity of ethylacetate extract of *Trigonella foenum-graecum* with different treatments

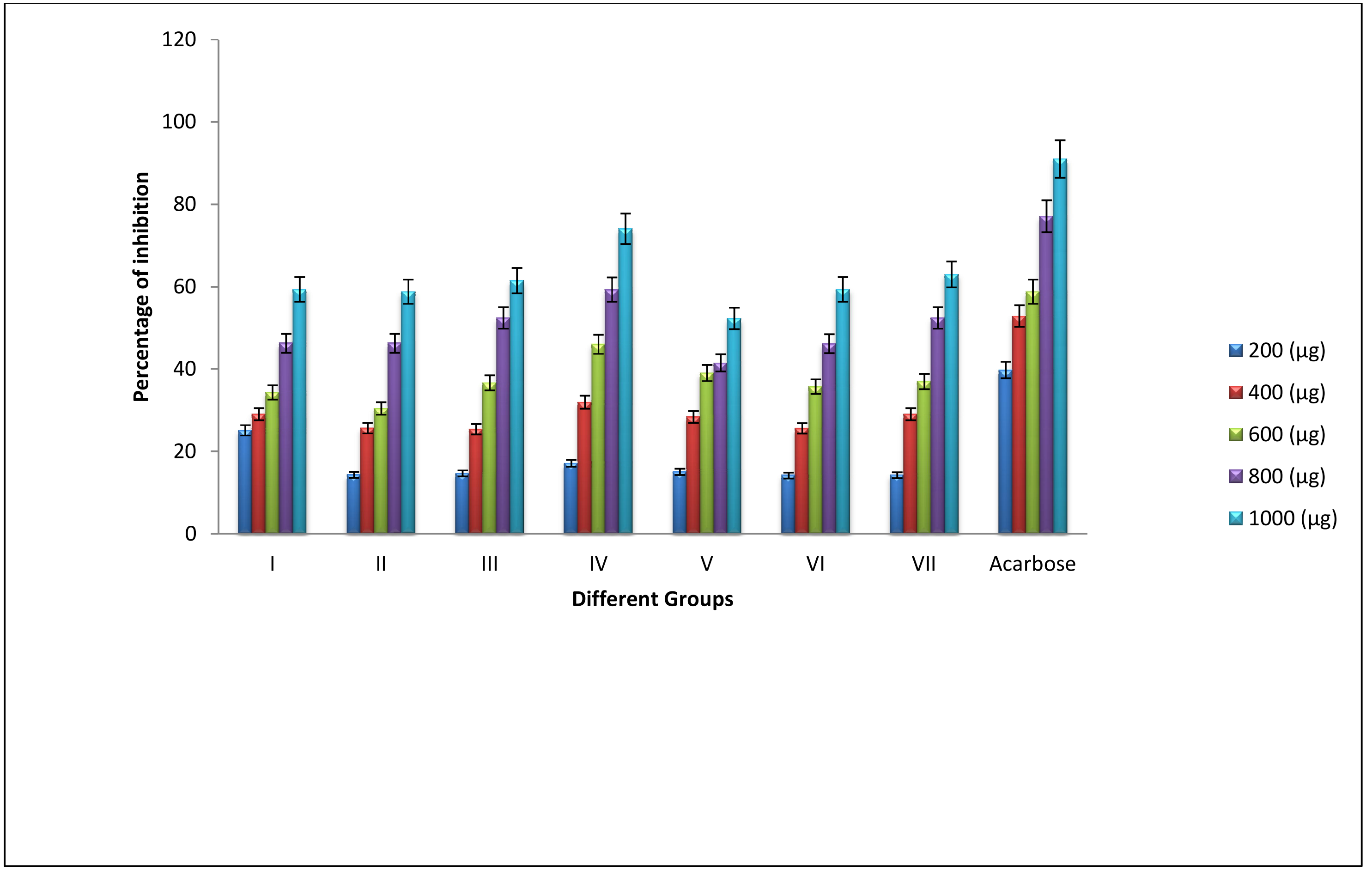

Fig. 2. DPPH scavenging activity of ethanol extract of *Trigonella foenum-graecum* with different treatments

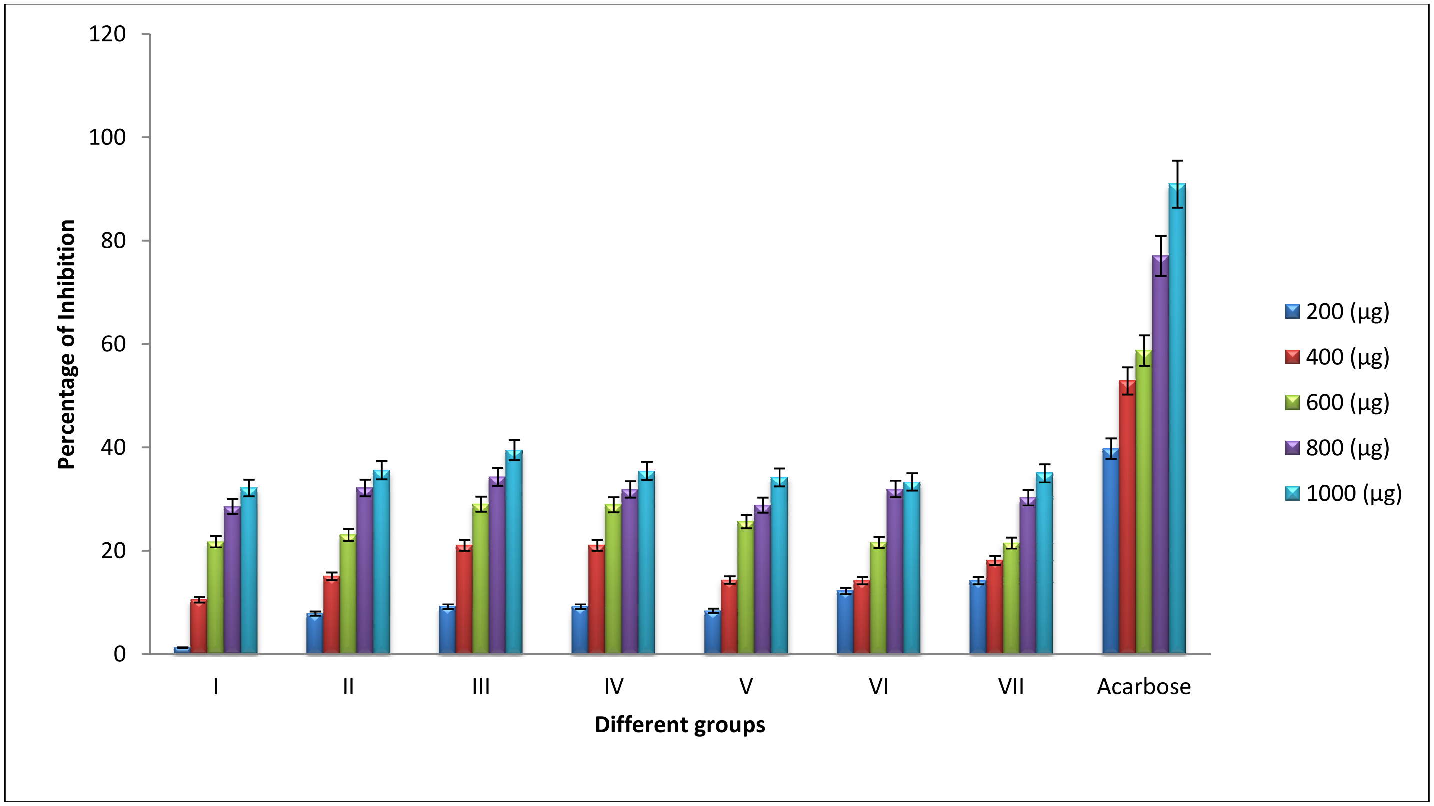

Fig. 3. DPPH scavenging activity of aqueous extract of *Trigonella foenum-graecum* with different treatments

the synergistic effects of different compounds. Many studies have shown that many polyphenols contribute significantly to the antioxidant activity [17,18] and act as highly effective free radical scavengers which are mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [19].

Out of which ethanol extracts of *Trigonella foenum-graecum* exhibits maximum level of DPPH free radical scavenging activity and group IV possessed the good activity when compared to other groups due to presence of vermicompost and *Lampito maurtii*. Also, Researchers are increasingly embracing green technology and the usage of sustainable and environmentally friendly approaches. Plants produce essential secondary metabolites for their adaptation and defense against environmental stressors. Our results are akin with Yusof et al. [20] reported the green technology and environmentally friendly (Vermicompost) approaches to enhance the production of valuable secondary metabolites in plants. The application of vermicompost as an alternative to chemical fertilizer not only produces healthier plants, but it also increases plant resistance toward pests and diseases [21]. Moreover, vermicomposting is a quicker and more cost-effective technique for composting as it helps in diverting organic waste from landfills.

4. CONCLUSION

Vermicompost treatment showed significant effect on the *Trigonella foenum- graecum* antioxidant activity and the composition of bioactive compounds. The usage of vermicompost produced plants with enhanced the bioactive compounds when compared to other groups. Ethanol extracts of *Trigonella foenum-graecum* exhibits maximum level of DPPH free radical scavenging activity and group IV (vermicompost and *Lampito maurtii*) possessed the good activity when compared to other groups. This implies that vermicompost cannot completely replace but could be used as an additional supplement to current fertilization practice to reduce environmental pollution and ensure agricultural sustainability. Further study is necessary for isolation and characterization of the active antioxidants, which may serve as a potential source of natural antioxidants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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