

ANTIOXIDANT IN VIVO, IN VITRO ACTIVITY ASSESSMENT AND ACUTE TOXICITY OF AQUEOUS EXTRACT OF GOMPHRENA CELOSIODES (AMARANTHACEAE)

ASBTRACT

Oxidative stress, an imbalance between the production of reactive oxygen species (ROS) and their cellular detoxification by antioxidants. ROS cause oxidation of membrane phospholipids, proteins and DNA

Many plants possess antioxidant ingredients that provided efficacy by additive or synergistic activities. *Gomphrena celosioides* (Amaranthaceae) was used for treatment of icterus, and malaria in Africa. In this study, we evaluated the in vivo, in vitro antioxidant activity and acute toxicity effects of *Gomphrena celosioides* (Gc). Acute toxicity study was undertaken on 48 rats using 100 to 4000 mg kg⁻¹ of Gc. In vivo antioxidant activity was determined, 20 Rabbits were divided into 4 groups of 5 animals. Group 1 to 2 received Gc 100-200 mg kg⁻¹ per by intraperitoneally during 3 days. Group 3 received vitamin C (10 mg kg⁻¹ per day) as a reference antioxidant for comparison. Group 4 was treated as control and received only saline (NaCl 0.9%). In each group, a blood sample was taken on the first day (J0) before the extract injection and then after extract injection at days 3 (J3), 6 (J6), 9 (J9) and 12 (J12).

The DL50 is 1000 mg/kg. The total phenolic contents = 3.44 ± 0.50 mg Gallic Acid Equivalent (GAE) /g content while flavonoid = 9.91 ± 0.45 mg Quercetin Equivalent (QE/g). The aqueous extracts of the plant showed strong free radical scavenging activity, reducing power and antioxidant activity. In vivo Gc possesses strong reducing power similar to vitamin C and lipid peroxidation inhibition effect to J6 and J9 and the vitamin C to J6.

This study in vitro suggests that the aqueous extract of Gc possesses a strong free radical scavenging activity, a low reducing power and a strong inhibition power of lipid peroxidation compared with the vitamin C. Furthermore in vivo, the reducing powers and inhibition of the lipid peroxidation of Gc were observed as the vitamin C. Finally, the weak toxicity and antioxidant activity of the Gc extract observed in vitro and vivo could be used in the management of inflammatory diseases and in the fight against stress oxidant.

Key words: Antioxidant activity, *Gomphrena celosioides*, Aqueous extracts, acute toxicity, Côte d'Ivoire.

1.INTRODUCTION

Health is one of the essential pillars of the man in his quest for his tireless and perpetual welfare. However in recent times we are seeing a recrudescence as well as the emergence of new forms of diseases such as AIDS, which weakens our immune system. The body is then subject to many opportunistic diseases such as skin cancer, tuberculosis, herpes zoster, diarrhea and encephalopathy. All diseases are mainly due to non-compliance with minimum hygiene rules and etiquette but also to the soaring prices of pharmaceuticals and increasing poverty^{1;2}. Indeed, our people are illiterate and often prefer to self-medicate using drugs they do not know the mode of action, biological targets, and side effects of the molecules with which they poison all day long. Thus, although the disease reached a critical phase sometimes causing serious damage which is sometimes irreversible when patients move towards health centers often late or they are moving towards traditional healers. According to Pousset³ and Sofowora⁴, people used plants for healing and this is certainly due to poverty but also to the richness of our flora in medicinal plants. On the basis of our rich flora, identified 761 species of medicinal drug^{5;6} has identified several species of medicinal plants in Africa. Our knowledge of the flora and therapeutic properties of our pharmacopoeia are based on a combination of traditional techniques without empirical scientific foundation for the most part reliable, medicinal plants are a source of new molecules with immunogenic activity and antioxidant properties exploitable^{7;8}. It is in this context that this study has served as interconnection between modern medicine and pharmacology for enhance it. It will ensure the safety of the use of

molecules isolated from plants and the development of new drugs designed to scientific methods. Hence, in line with the current trend of finding naturally occurring antioxidants, this study was designed to evaluate *in vitro* and *in vivo* antioxidant activity and acute toxicity of aqueous extract of *Gomphrena celosioides* (Amaranthaceae) in order to justify its ethnomedicinal importance.

2. Materials and Methods

2.1 Chemicals

All chemicals were of highest purity ($\geq 99.0\%$). Quercetin was purchased from SigmaChemical Co. (St., Louis, USA). Gallic acid, Folin-Ciocalteu reagent and methanol were from Merck Co. (Germany). Sodium acetate, 2,4,6-tripyridyl-striazine (TPTZ), 2-thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropan (MDA), trichloroacetic acid (TCA), glacial acetic acid, $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$, HCl, and n-butyl alcohol were purchased from Merck Germany.

2.2 Extract Preparation

Gomphrena celosioides plants used for this study were collected to the herbarium of "Centre National de Floristique" of the University of Cocody-Abidjan (Cote D'Ivoire) in April 2010. The plants were identified and authenticated by Professor AKE ASSI at the Department of Botany, University of Cocody. The plants were dried at room temperature and ground in a grinder (IKAMAG RCT®). 100 g of *Gomphrena celosioides* powder was extracted in 1000 mL of distilled water by maceration for 48 hours. The solvent was removed under vacuum at temperature below 50°C and the extracts freeze-dried. Thereafter, the extract was filtered and the filtrate stored at -20°C when not in use. The mixture was filtered using Whatman No. 1. The dried nuts were ground into powder and allowed for 2 Weeks days.

2.3. In Vitro

2.3.1 Total phenols determination

Total phenols were determined by Folin-Ciocalteu reagent⁹. A diluted aliquot of each plant extract (0.5 mL of 0.1 g/mL) or gallic acid (standard phenolic compound) was mixed with Folin-Ciocalteu reagent (5 mL, 1/10 diluted with distilled water) and aqueous Na_2CO_3 (4mL, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by spectrophotometer at 765 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of sample, using a standard curve generated with gallic acid.

2.3.2 Total flavonoids determination

Aluminium chloride colorimetric method was used for flavonoids determination¹⁰. Each plant extract (0.5 ml of 0.1 g/mL) in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of aluminium chloride (10%), 0.1 mL of potassium acetate (1 M) and 2.8 mL of distilled water. It remained at room temperature for 30 min. Absorbance of the reaction mixture was measured at 415 nm. The absorbance of a prepared blank was also recorded. Total flavonoid contents expressed as quercetin equivalents (QE) in micrograms per gram dry weight of extract were also determined using the standard curve of quercetin.

2.3.3 Free radical scavenging activities Determination

The free radical scavenging activity of the extracts and vitamin C were measured with the DPPH method. This spectrophotometric assay uses stable radical DPPH as a reagent¹¹. Thus, 2.5 mL of various concentrations using serial 2-fold dilutions of the extracts and vitamin C in methanol was added to 2.5 mL of the solution of DPPH (0.02 mg/mL). After 15 mn incubation period at room temperature,

absorbance at 517 nm was determined after 15 mn, and the percent inhibition activity was calculated as $[(A0-A1)/A0] \times 100$, where A0 was the absorbance of the control, and A1 was the absorbance of the extract/standard.

2.3.4 Reducing power determination

The determination of the reducing power was conducted according to the method developed by Oyaizu¹² for the reducing power test. The solution of plant extracts (1 mL, 0 - 10 mg/mL) was spiked with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of potassium ferricyanide (1%). The mixture was then placed in a 50°C water bath for 20 minutes. After cooling rapidly, 1 mL of 10 percent trichloroacetic acid (10%) was added and centrifuged at 3000 rpm for 10 minutes. The supernatant (1 mL) was then mixed with 2 mL of distilled water and 0.1 mL of ferric chloride (0.1%). The absorbance at 700 nm was recorded for the reaction for 10 minutes. Increasing absorbance of the reaction mixture indicated an increase of the reducing power.

2.3.5 Inhibition lipid peroxidation determination

The procedure of using a Fenton reaction-induced lipid peroxidation has been adapted for this assay¹³. One (01) mL of extracts of all species in concentrations of 600 mg/mL have been mixed with 300 mL of Tris-HCl buffer (pH 7.5), 500 mL of linoleic acid (20 mM) and 100 mL of FeSO₄ (4 mM). The peroxidation was started with addition of 100 mL of vitamin C (5 mM). The reaction mixture was incubated at 37 °C for 60 min. After, 2 mL of ice cold trichloroacetic acid (10%) was added and 1 ml aliquots of the samples were added with 1 mL of thiobarbituric acid (1%). The mixture was heated in a water bath at 95 °C for 60 min. The absorbance was determined at 532 nm. Gallic acid was used as standard. The inhibitory percentage of lipid peroxidation was calculated $(1 - \text{sample absorbance} / \text{control absorbance}) \times 100$.

2.4 In Vivo

2.4.1 Experimental design

All the animals' experimental procedures were conducted after the approval of the Ethical Guidelines of University (Côte d'Ivoire) Committee on Animal Resources. They were in strict accordance with the guidelines for Care and Use of Laboratory Animals (National Academy of Sciences, 1996) and the statements of the European Union regarding the handling of experimental animals (86/609/EEC)¹⁴.

2.4.2 Group designing for acute oral toxicity study

48 rats (weighing between 65-120 g) were used for acute toxicity. Rats were divided into 8 groups of 6 animals. They were placed in cages in a room at a constant temperature of $22 \pm 2^\circ\text{C}$ with 12-h light and dark cycles and fed standard pellet diet and water ad libitum. Toxicity study was undertaken on 48 rats using 100, 500, 1000, 2000 and 4000 mg kg⁻¹ of *Gomphrena celosioides* which administered by intraperitoneal and animals receiving saline 0.9% served as control. The signs and symptoms associated with the *Gomphrena celosioides* administration were observed at 0, 30, 60, 120, 180 and 240 min after and then once a day for the next 5 days. At the end of the period, the number of survivors was recorded and the acute toxicological effect was estimated through the acute toxic class method¹⁵ to find out the safe dose.

2.4.3 Group designing for antioxidant activity study

Rabbits of the species *Oryctolagus cuniculus*, aged between 3 and 4 months and weighing 1.5 ± 0.3 kg were used. They were provided by a breeding company and were acclimatized at 25 °C in a 12h light/12 h dark cycle in the pet room of the laboratory for 14 days prior to

the experiment. The animals were given feed (FACI ® Company, Abidjan, Côte d'Ivoire) for rabbits and tap water ad libitum. After the acute toxicity study, 20 Rabbits were divided into 4 groups of 5 animals. The extract was dissolved in normal saline to provide a solution. Animals from group 1 to 2 received the extract dry, namely 100 and 200 mg kg⁻¹ per by intraperitoneal during 3 days. Group 3 received vitamin C (10 mg kg⁻¹ per day) dissolved in saline by intraperitoneal as a reference antioxidant for comparison. The group 4 of animals was treated as control and received only saline (NaCl 0.9%). In each group, a blood sample was taken from the marginal vein of the rabbit ear on the first day (J0) before the extract injection and then after extract injection at days 3(J3), 6 (J6), (J9) and 12 (J12). The blood was centrifuged at 2000 × g for 10 minutes to separate serum. The serum was kept at -20 °C for subsequent determination of lipid peroxidation and antioxidant status.

2.4.4 Antioxidant activity methods

2.4.4.1 Lipid peroxidation assay

Thiobarbituric Acid Reactive Substances (TBARS) assay is the method of choice for screening and monitoring lipid peroxidation, a major indicator of oxidative stress. To precipitate the serum proteins, 2.5 mL of TCA 20% (m/V) was added into 0.5 mL of the sample, which was then centrifuged at 1500 × g for 10 min. Then 2.5 mL of sulfuric acid (0.05 m L⁻¹) and 2 mL TBA (0.2%) was added to the sediment, shaken, and incubated for 30 min in a boiling water bath. Then, 4 mL n-butanol was added, and the solution was centrifuged, cooled and the supernatant absorption was recorded at 532 nm using a UV-Visible spectrophotometer (Shimadzu, Japan). The calibration curve was obtained using different concentrations of 1,1,3,3-tetramethoxypropane as standard to determine the concentration of TBA-MDA adducts in samples¹⁶.

2.4.4.2 Total antioxidant power (TAP) assay

The total antioxidant capacity of serum was determined by measuring its ability to reduce Fe³⁺ to Fe²⁺ by the FRAP (Ferric Reducing Ability of Plasma) test. The FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe(II)-tripirydyltriazine compound from Fe(III) by the action of electron donating antioxidants. The FRAP reagent consists of 300 mmol L⁻¹ acetate buffer pH = 3.6, 10 mmol L⁻¹ TPTZ in 40 mmol L⁻¹ HCl and 20 mmol L⁻¹ FeCl₃·6 H₂O in the ratio of 10:1:1. Briefly, 10 mL of serum was added to 300 mL freshly prepared and prewarmed (37 °C) FRAP reagent in a test tube and incubated at 37 °C for 10 min. The absorbance of the blue colored complex was read against a reagent blank (300 mL FRAP reagent + 10 mL distilled water) at 593 nm. Standard solutions of Fe²⁺ in the range of 100 to 1000 mmol L⁻¹ were prepared from ferrous sulphate (FeSO₄·7 H₂O) in water. The data was expressed as mmol ferric ions reduced to ferrous form per liter (FRAP value)¹⁷.

2.4.5 Statistical analysis

Data obtained are presented as means (±S.E.M.). The differences between the data obtained were subjected to one-way analysis of variance (ANOVA; 95 % confidence interval), followed by Dunnett's test. In all cases, statistical significance was established at values of P ≤ 0.05.

3. Results

3.1. Determination of Acute Toxicity,

The intraperitoneally administration of *Gomphrena celosioides* at the dose 100 mg/kg did not exhibit death and any signs of toxicity up to 14 days. Indeed, the DL₅₀ is 1000 mg/kg (LD₅₀ > 100 mg/kg). The acute toxicity test (LD₅₀) demonstrated that *Gomphrena celosioides* extract is not lethal up to a dose of 100 mg kg⁻¹.

3.2. Determination of antioxidant activity

3.2.1. In vitro

3.2.1.1 Total phenols determination

The total phenols of *Gomphrena celosioides* has been determined and the value is 3.44 ± 0.50 mg gallic acid equivalent /g of *Gomphrena celosioides*

3.2.1.2 Total flavonoids determination

The total flavonoids of *Gomphrena celosioides* is 9.91 ± 0.45 mg quercetin/g of *Gomphrena celosioides*

3.2.1.3 Free radical scavenging activities determination

The free radical scavenging of *Gomphrena celosioides* (CI_{50}) is 3.5 ± 0.5 μ g/mL and the Vitamin C $CI_{50} = 87 \pm 1.5$ μ g/mL

3.2.1.4 Reducing power of the aqueous extract of *Gomphrena celosioides*

The results of the reducing activity of *Gomphrena celosioides* aqueous extract and vitamin C are presented by the figure 1. In the figure 1, the very strong slope of vitamin C and the low slope for *Gomphrena celosioides*, indicates that the reducing power of *Gomphrena celosioides* is lower than the vitamin C.

3.2.1.5 lipids peroxydation inhibition

The results of the lipids peroxydation inhibition by the aqueous extract of *Gomphrena celosioides* and the Gallic acid expressed in percent are presented by the following figure 2:

The inhibition of lipid peroxidation the cola extract and Gallic acid to the concentration 125 and 500 μ g/mL are respectively: Extract of *Gomphrena celosioides*: 16.18 ± 1.27 and 48.50 ± 4.74 %; Gallic acid: 13.10 ± 2.73 and 34.12 ± 4.13 %

The aqueous extract of *Gomphrena celosioides* have been an antioxidant activity more elevated than the Gallic acid, a reference molecule.

3.2.2 In vivo

3.2.2.1 lipid peroxidation Inhibition determination (TBARS method)

The results of the antioxidant activity of the aqueous total extract of *Gomphrena celosioides* and the vitamin C are represented in percent of lipid peroxydation inhibition in figure 3. The figure 3 reveals that there was no significant difference J0, J3, J6, J9 and J12 at the normal control (NaCl). The inhibition was observed at the vitamin C to J6 and the *Gomphrena celosioides* aqueous total extract to J6 and J9.

3.2.2.2 Total antioxidant power determination of *Gomphrena celosioides*

The results of the total antioxidant power activity of the aqueous total extract of *Gomphrena celosioides* and the vitamin C are represented by the figure4. There was no significant difference in the normal control (NaCl) to J0, J3, J6, J9 and J12. The total antioxidant

power activity of the vitamin C increases at J0 to J6 and decreases at J9 to J12. Concerning the aqueous total extract of *Gomphrena celosioides*, an increase total antioxidant power at J3 to J9 and a decrease for both concentrations of the extract to J12 was observed.

4. Discussion

The acute toxicity study of the aqueous extract total *Gomphrena celosioides* (EAG) in rats was used to determine the LD50 equal 1000 mg / kg, when the extract was administered intraperitoneally. The value of 1000 mg / kg as a slightly toxic substance¹⁷ that the EAG orally is almost not toxic. However, when administered intraperitoneally, it is slightly toxic.

Celosioides extracts exhibited potent in vitro antioxidant activity in determination of Polyphenols, DPPH-radical scavenging assay, reducing power and lipids peroxydation inhibition in comparison to the known antioxidants, such as vitamin C.

Polyphenols are the major plant compounds in the free radical scavenging and antioxidant activity¹⁸. This activity is mainly due to their redox properties¹⁹ which allow them to act as reducing agents, hydrogen donors, singlet and triplet oxygen quenchers or decomposing peroxides agents.

The study indicates that aqueous extracts of *Gomphrena celosioides* have possessed phenolic compounds and flavonoids. In the DPPH test provided information on the reactivity of test compounds with a stable free radical. The efficacies of *Gomphrena celosioides* are often associated with their ability to scavenge stable free radicals²⁰.

The scavenging activity of the extract of *Gomphrena celosioides* was as strong as that of vitamin C and was positively correlated with its highest level of phenolic compounds²¹.

The reducing power is widely used to evaluate the antioxidant activity of plants extracts. Earlier authors have observed a direct correlation between antioxidant activities and reducing power of certain plant extracts²². Our data on the reducing power of the extracts of plants indicated that *Gomphrena celosioides* possesses strong reducing power similar to vitamin C.

Lipid peroxidation is a complex process in which the oxidation of polyunsaturated fatty acids of membrane lipids leads to membrane damage and cell death²³. From the result of our investigation, *Gomphrena celosioides* has the potential to prevent this cell death due to lipid peroxidation by inhibiting the lipid peroxidation process.

Our results suggest the possibility that *Gomphrena celosioides* is useful for prevention of the phenomenon of the oxidative stress. However, *Gomphrena celosioides* extracts exhibited high inhibitory effects to lipid peroxidation of linoleic acid. The activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging. High inhibitory effect of lipid peroxidation of the extracts could be due to the abundant presence of antioxidant active compounds^{24; 25}.

The *Gomphrena celosioides* lipid peroxidation inhibition effect is observed to J6 and J9 and the vitamin C to J6. This finding supported reports in the literature that vitamin C is a strong reducer of MDA concentration. Vitamin C can protect the cell membrane and cytosolic component of cells against the damage of oxidant²⁵. Indeed, the phytochemical study of the *Gomphrena celosioides* have showed Flavonoids, saponins, sterols, terpenes, tannins and Coumarins contains²⁵. This is a likely proof of its antioxidative properties which has high inhibition of lipid peroxidation. This may further be strengthened by the result obtained for MDA (lipid peroxidation) determination, where the values for the test is significantly reduced compared with the normal control (NaCl) values at J6 and J9.

Gomphrena celosioides administration caused a decrease in the levels of MDA is in accordance with the findings since marked decrease in the levels of lipid peroxidation was determined in rabbit. MDA is a product of lipid peroxidation^{26;27}. An increase in the blood MDA levels is an indication of elevated level of lipid peroxidation. Indeed, Yagi in 1986 demonstrated that serum TBARS are mainly contained in LDL²⁸ namely, a decrease of TBARS in plasma suggests that Gomphrena celosioides would prevent the conversion of native LDL to oxidized LDL. It is well established that extensive lipid peroxidation leads to disorganization of membrane by peroxidation of unsaturated fatty acids which also alters the ratio of polyunsaturated to other fatty acids. This would lead to a decrease in the membrane fluidity and the death of the cell according to Devaki et al.²⁸. From the result of our investigation, Gomphrena celosioides has the potential to prevent this cell death due to lipid peroxidation by inhibiting the lipid peroxidation process.

Our results suggest the possibility that Gomphrena celosioides is useful for prevention of the phenomenon of the oxidative stress. This is that Gomphrena celosioides showed antioxidant activity like Vitamin C in vitro. It is well known that green tea contains a large amount of phenolic compounds, and among these compounds catechin seems to play an outstanding role in the antioxidant activity²⁹.

5. CONCLUSION

In conclusion, this study was designed to investigate the phenolic contents and evaluate the in vitro and in vitro antioxidant activities of Gomphrena celosioides. Phenolic compounds, flavonoids, free radical scavenging, reducing power and lipid peroxydation inhibition were founded in aqueous extracts of Gomphrena celosioides. These activities may possibly due to presence of biomarker compounds such as gallic acid and quercetin in this plant which indirectly helped to decrease the levels of MDA, prevent the alteration of lipids level and increase antioxidant status in rabbit. The various biological activities of Gomphrena celosioides could be interest for the food industry and for preventive medicine. Further work is needed to determine the preventive effects of Gomphrena celosioides against various diseases caused by oxidative damage. The weak toxicity of Gomphrena celosioides improves the ethnopharmacological knowledge, which use of fruit as an economically viable source of natural and potent antioxidant

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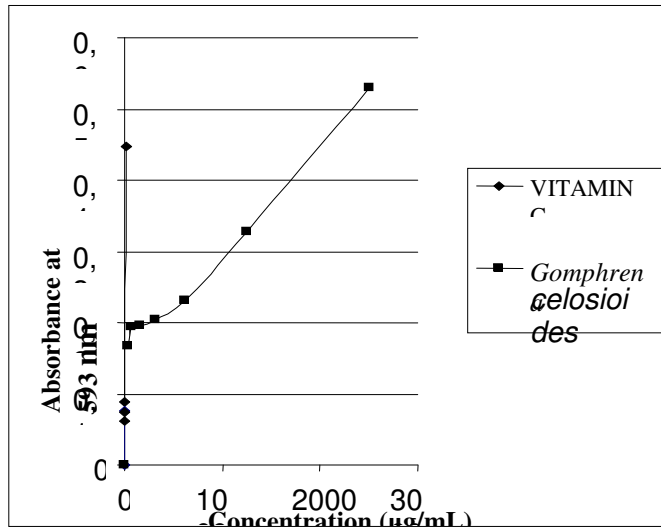


Figure 1: Determination of the reducing power of Gomphrena celosioides and Vitamin C

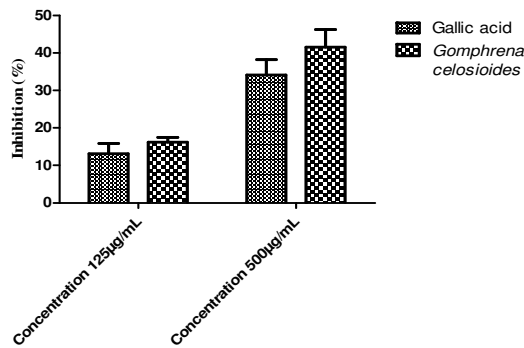


Figure 2: Lipids peroxydation inhibition by the aqueous extract of Gomphrena celosioides and the Gallic acid

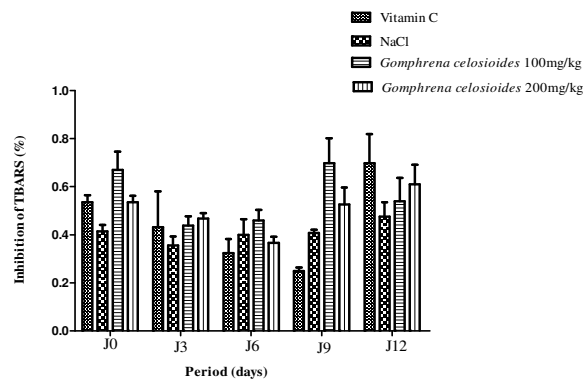


Figure 3: lipid peroxidation Inhibition of *Gomphrena celosioides* by the TBARS method

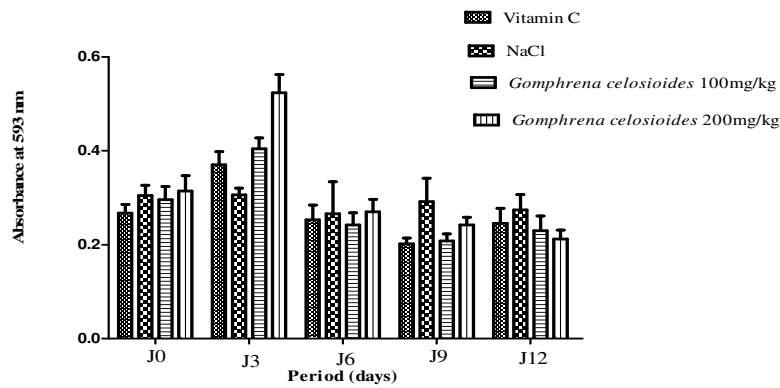


Figure 4: Total antioxidant power determination of *Gomphrena celosioides*

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