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In-vitro analysis of Antioxidant and Anti-Inflammatory activity of crude seed extract of *Amaranthus caudatus*: An Ethnobotanical Plant

Dr. Thoyajaksha, Minakshi Pandey D., Deepak R. Chandra*, Rakhi Bajpai

Department of Botany, Maharani College for Women, Mysore, Karanataka

*Research and Development Centre, Bharathiar University, Coimbatore,

Department of Biotechnology, GPS Institute of Agricultural Management, recognised by Bangalore University, Bengaluru, Karnataka, India

School of Life & Allied Sciences, ITM University, Raipur, Chhattisgarh

***Address For correspondence**

***Corresponding Author:** Deepak R Chandra

Address: Research and Development Center, Bharathiar University, Coimbatore, Tamilnadu, India

Email: deepak.stanley@gmail.com

Abstract

Numerous diseases, including cancer, have been linked to inflammation, and the treatments that are currently available have side effects. The anti-inflammatory properties of members of the family Amaranthaceae have been demonstrated. The effectiveness of *Amaranthus caudatus*'s in-vitro anti-inflammatory and antioxidant properties was evaluated in this study. This study looked into the seed ethanolic extract of *Amaranthus caudatus*'s phytochemical, antioxidant, and anti-inflammatory properties. Various chemical tests for phytochemical components were performed on the extract; The in-vitro DPPH radical scavenging activity assay was used to measure their antioxidant activity, and the protein denaturation inhibition method was used to measure their anti-inflammatory activity. Carbohydrates, alkaloids, flavonoids, tannins, and phlobatannins were found in extracts after phytochemical testing. Anti-inflammatory studies showed that diclofenac effective concentration (EC50) of 275.19 µg/ml which was higher than EC50 of ethanolic seed extract of 518.92 µg/ml. The effective concentration (EC50) of ascorbic acid was 288.75 µg/ml, which was

lower than the EC50 of ethanolic seed extract of 114.25 μg/ml. This study revealed that ethanolic seed extract of *Amaranthus caudatus* possess anti-oxidant activity against free radicals scavenging in DPPH method and can act as an effective anti-oxidant. The anti-inflammatory effects that inhibit the protein denaturation method.

Keywords: *Amaranthus caudatus*, seed extract, phytochemicals, anti-inflammatory, anti-oxidant.

Introduction

Amaranthus spp., or amaranth, is a gluten-free pseudocereal that thrives in all temperate-tropical regions of the world. It is primarily grown in Mexico and South America. There are approximately 70 species of Amaranthus found worldwide in temperate, subtropical, and tropical climate zones. According to Rastogi & Shukla (2013), a few of them are distributed worldwide. India is home to approximately 20 cultivated and wild species. According to Grubben & Van Stolen (1981), some species of grain are thought to be native to South and Central America, while others are found in Europe, Asia, Africa, and Australia. In its own nation of beginning, seed Amaranthus (A. caudatus) is realized by different names like kiwicha. According to Rastogi & Shukla (2013), India is one of the distribution centers of amaranth, with the other being tropical America. All the more as of late, it has been proposed that China is the world's biggest maker of amaranth, both for grain utilization and feed plant use. Other significant amaranth producing nations are the US, Canada and Argentina (Coelho et al., 2018). A few years ago, the agro-industrial uses of amaranth seed were described, as was the first result of research into its processing, utilization, and transformation into high-quality infant food. In comparison to the majority of cereals, amaranths produce significant quantities of edible seeds that are richer in minerals and vitamins (Achigan-Dako et al., 2014, Sanchez-Marroquin et al., 1986, Sanchez-Marroquin, 1983). Amaranth, which is regarded as the seed of the twenty-first century, is a prime example (Vetter, 1994; Zheleznov, Solonenko, & Zheleznova, 1997). Amaranthus is an essential food crop. It is a rare plant whose seeds are used to make cereal and its leaves are eaten as vegetables.

Amaranthus seeds are used as grain in Bolivia and Peru. Molasses and candies are made from popped seeds. In Peru, the seeds are popped and used to make flour or bells that are bound with syrup. In India, laddoos primarily consist of seeds, which are sometimes consumed alongside rice after being boiled. Chapatis are made from seed flour in the Himalayas, and satto is made from seed flour in Nepal. The seeds are used to make crackers, cookies, cereal, baked goods, and other baked goods in the United States. A customary utilization of amaranth in Mexico and different nations is to blend popped amaranth in with honey to make a kind of café or nibble cake. Whole seeds are sometimes used in porridge, as a condiment, or in other dishes. The technological and functional properties of wheat and other grain products can be significantly altered by substituting amaranth for them. According to Singhal and Kulkarni's 1988b and 1988a studies, amaranth grain starch maize could be used to thicken sauces. Additionally, it has been reported that, as an alternative to amaranth flour, expanded amaranth grains (10–20 percent) could boost the bread's nutritional value by adding more iron, phosphorus, calcium, magnesium, and potassium (Bodroza-Solarov et al., 2008, Coelho et al., 2018, Pasko et al., 2007, Shukla et al., 2006).

According to Kalinova and Dadakova (2009), Pasko et al. (2011), a significant amount of phenolic acids and flavonoids from A. caudatus seeds exhibit antioxidant activity. The biological activities of phenolic compounds, which have the potential to be free radical scavengers and antioxidants, have been the subject of numerous studies. It was found that a methanolic concentrate of A. caudatus contain 48% all out phenolic portion (Kumar et al., 2011). According to Kumar et al., (2011) and Peiretti et al., (2017) these extracts had effective scavenging properties and strong antioxidant activity. *Amaranthus caudatus* seeds are rich in proteins, which comprise up to 15-43% and 14-30% of freshmatter, individually. *Amaranthus caudatus* seed proteins have good functional properties, a high bioavailability, and a balanced amino acid composition. Amaranth seeds contain dietary fiber, vitamins and their precursors (ascorbic acid, riboflavin, tocols, carotenoids), as well as minerals (Ca, Fe, Mg, K, Cu, Zn, and Mn).

The purpose of this research was to determine whether ethanolic seed extract of *A. caudatus* could be used as a source of antioxidants for food production.

Materials & Methodology

Chemicals Used

Ethanol (100 %), Bovine Serum Albumin (pH 6-7) (SRL, Chennai), Phosphate Buffer, Ascorbic acid (SRL, Mumbai), DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) (SRL, Mumbai), Distilled water and Diclofenac sodium (Control Drug) were used.

Collection and Identification Plant Material

The seeds of the *Amaranthus caudatus* variety was gathered from a public retail market in the city of Mysuru, Karnataka, India for experimental investigation. The specimen seeds were identified by Thoyajaksha, Botanist, Department of Botany, Maharani College, Mysuru, India.

Crude Extraction from Seeds

In this section of the study, a modified approach as described by James et al., (2013) was utilized. *Amaranthus caudatus* seed was air-dried for 7 days under shaded sunlight. The air-dried seeds were powdered using mixer until it becomes fine. The grounded powder was weighed and stored in air-tight ziplock covers. The maceration method was followed for crude extraction in room condition for 2 days. 5 grams of powdered *Amaranthus caudatus* seed was added to 100 ml of ethanol (100 %) and left undisturbed for 2 days. After that, the extract was filtered using whatman filter paper no.1. The recovered filtrate was concentrated and weighed. The stock of 1 mg/ml crude extract was diluted with DMSO to yield five varied concentration (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml and 62.5 µg/ml).

Qualitative Phytochemicals Assay

The ethanolic seed extract of *Amaranthus caudatus* were subjected to qualitative phytochemical screening in accordance with Evans WC (1996) standard methods in order to ascertain the presence of phytochemicals. The respective solvent (ethanol) used for their extraction were dissolved in 50 mL of the seed extract (5 mg).

Carbohydrates, free reducing sugar, anthracene derivatives, cardiac glycosides, saponin, tannin, flavonoids, alkaloids, unsaturated sterols, and triterpenes were prepared for qualitative phytochemical analysis.

Anti-Inflammatory Activity by Protein Denaturation Method

According to protocol in (Khalid et al., 2020), the anti-inflammatory activity was performed. The reaction mixture contains 0.2 ml of bovine serum albumin (from Sigma aldrich, Mumbai), 2.8 ml of phosphate buffer (pH 6.4) and 2 ml of *Amaranthus caudatus* seed ethanolic crude extract of various concentration (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml and 62.5 µg/ml). Same volume of distilled water served as blank. Then the reaction mixtures were incubated at room temperature for 15 min and then heated at 95 °C for 5 min using water bath. After heating, the reaction mixture was cooled down to room temperature. The UV absorbance was measured at 660 nm (LCD LABMAN Visible Spectrophotometer LMSP-V320). The control drug Diclofenac sodium was utilized at the final concentrations of (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml and 62.5 µg/ml) were used as comparison. The percentage of anti-inflammatory activity by inhibition of protein denaturation was calculated by using the following formula:

% Anti-inflammatory activity = Absorbance of Control - Absorbance of test sample / Absorbance of Control × 100

Anti-Oxidant Activity by DPPH Method

The anti-oxidant activity was performed by following, Moraes-de-Souza et al. (2008) determined the DPPH radical scavenging activity with a few alterations. 0.5 mL of *Amaranthus caudatus* seed ethanolic crude extract of varied concentration (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml and 62.5 µg/ml) and 0.8 mL of DPPH radical solution containing 0.1 mM in ethanol formed the reaction mixture. It is reduced when DPPH reacts with an antioxidant that can donate hydrogen. A visible spectrophotometer (LCD LABMAN Visible Spectrophotometer LMSP-V320) was used to measure absorbance at 517 nm following a 30-minute incubation period.

The following equation was used to determine the antioxidant activity. The control drug Ascorbic acid was utilized at the final concentrations of (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml and 62.5 µg/ml) were used as comparison. The percentage of anti-oxidant activity by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging method and scavenging percentage was calculated by using the following formula:

% Anti-oxidant activity = Absorbance of Control - Absorbance of levetiracetam / Absorbance of Control × 100

Results

Amaranth contains secondary plant metabolites, which may play a significant role in the human diet due to their potential health benefits, in addition to macro- and micronutrients. The food value of natural compounds extracted from plants, such as storage lipids, essential oils, flavonoids, polyphenols, and pharmaceutics, has been extensively studied. These natural compounds are utilized as precursors by the cosmetics and pharmaceutical industries.

Figure No.1: *Amaranthus caudatus* seed ethanolic crude extract

Qualitative Phytochemicals Assay

The qualitative determination of phytochemicals in ethanol extract of *Amaranthus caudatus* seeds. The qualitative determination of phytochemicals present in the ethanol seed extract of Amaranthus caudatus is presented in Table 1. Result showed carbohydrates, Alkaloids, Flavonoids, Tannins, and Phlobatannins were present in the extract. Saponins and terpenoids were absent in the ethanolic extract.

Table 1: Phytochemicals found in ethanol extract of *Amaranthus caudatus* seeds

S.No.	Phytochemicals	Observation
1.	Alkaloids	Positive
2.	Flavonoids	Positive
3.	Tannins	Positive
4.	Phlobatannins	Positive
5.	Saponins	Negative
6.	Terpenoids	Negative
7.	Carbohydrates	Positive

In-vitro Anti-inflammatory activity

An In-vitro anti-inflammatory activity of *Amaranthus caudatus* seed ethanolic crude extract was studied by following Bovine Serum Albumin (BSA) denaturation method. Bovine serum albumin (BSA) in vitro anti-denaturation effects are proposed as a screening assay for the early stages of the drug discovery process, without the use of animals, for the detection of anti-inflammatory compounds (Williams et al., 2008). The percentage of denaturation inhibition by *Amaranthus caudatus* seed ethanolic crude extract and diclofenac with varied concentration (1000 μg/ml, 500 μg/ml, 250 μg/ml, 125 μg/ml and 62.5 μg/ml) shown in Table 2 and 3. The figure 2 and 3

represents the inhibitory effect of *Amaranthus caudatus* seed ethanolic crude extract and diclofenac drug effectively.

Table No.2: Percentage of Protein Denaturation Inhibition by *Amaranthus caudatus* seed ethanolic crude extract

***Amaranthus caudatus* seed ethanolic crude extract**	**% of Inhibition**
1000 µg/ml	89.69
500 µg/ml	55.81
250 µg/ml	40
125 µg/ml	25.81
62.5 µg/ml	20.98

Table No.3: Percentage of Protein Denaturation Inhibition by Diclofenac (Control Drug)

Diclofenac	**% of Inhibition**
1000 µg/ml	100
500 µg/ml	93.88
250 µg/ml	58.39
125 µg/ml	31.62
62.5 µg/ml	30.65

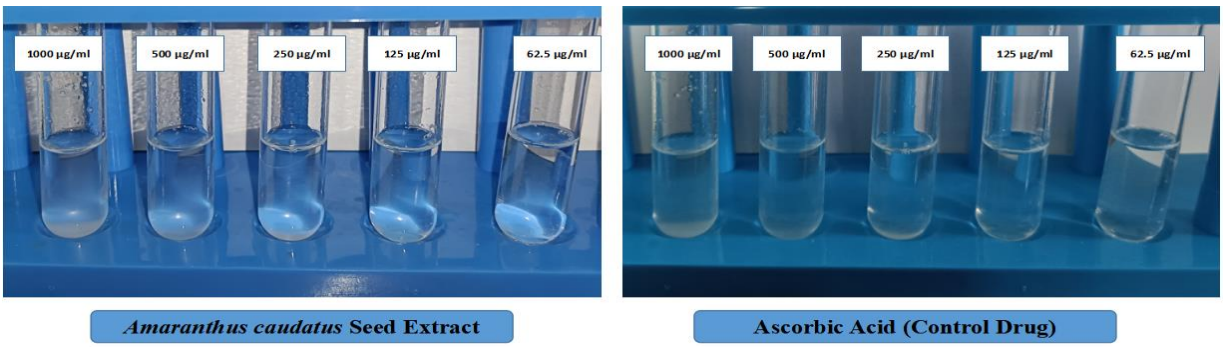

Figure No.2: Anti-Inflammatory Activity of *Amaranthus caudatus* seed ethanolic crude extract and Diclofenac (Control Drug)

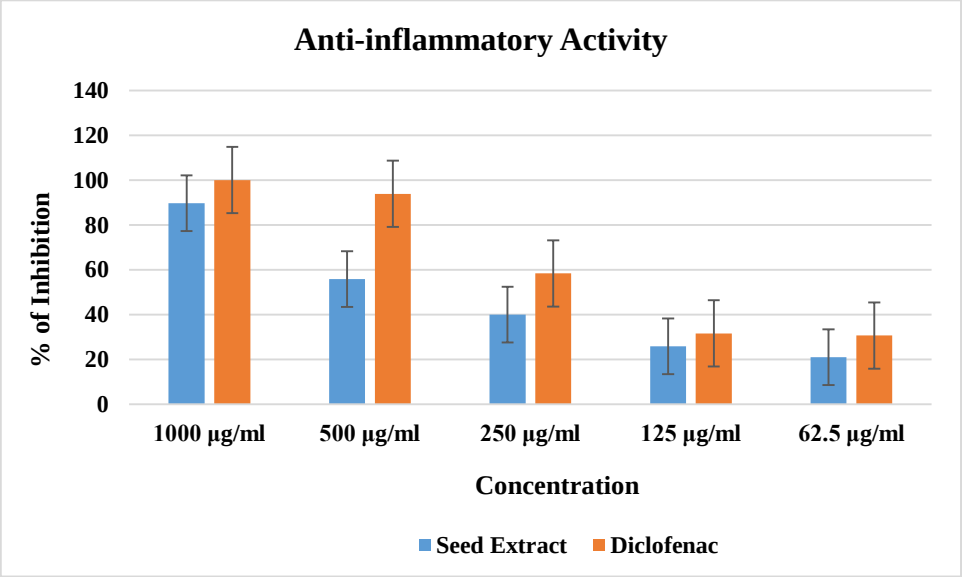

Figure No.3: Anti-Inflammatory Activity of *Amaranthus caudatus* seed ethanolic crude extract and Diclofenac (Control Drug)

Anti-Oxidant Activity by DPPH Method

The first method for determining a compound antioxidant activity is the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging method. The simplest method involves mixing the potential compound with DPPH solution and recording the absorbance after a predetermined time period (Kedare and Singh, 2011). The results are represented in (Table 4 and 5) and (Figure 4 and 5). Our study shown, the concentration of 1000 µg/ml, 500 µg/ml exhibited highest anti-oxidant activity.

The antioxidant activity of the seed extract of Amaranthus caudatus is shown in a plot of percentage inhibition of free radicals versus extract concentration based on their ability to scavenge on DPPH, a stable purple radical, and convert it into yellow diphenylhydrazine (Figure 4 and 5). Based on a linear regression of the percentage inhibition versus extract concentration, the antioxidant potential is inversely proportional to the effective concentration (EC50) value (Figure 6).

Ethanol extract had the highest percentage scavenging activity at all concentrations, but it was significantly ($p < 0.05$) lower than the scavenging activity of vitamin C (Ascorbic acid) at 250 µg/ml, 500 µg/ml, and 1000 µg/ml, respectively, as demonstrated by the percentage free radical scavenging activity of the seed extract.

Table No.4: Percentage of Inhibition by *Amaranthus caudatus* seed ethanolic crude extract

***Amaranthus caudatus* seed ethanolic crude extract**	**% of Inhibition**
1000 µg/ml	72
500 µg/ml	62
250 µg/ml	56
125 µg/ml	49
62.5 µg/ml	11

Table No.5: Percentage of Inhibition by Ascorbic acid (Control Drug)

Diclofenac	**% of Inhibition**
1000 µg/ml	83
500 µg/ml	74
250 µg/ml	73
125 µg/ml	67
62.5 µg/ml	64

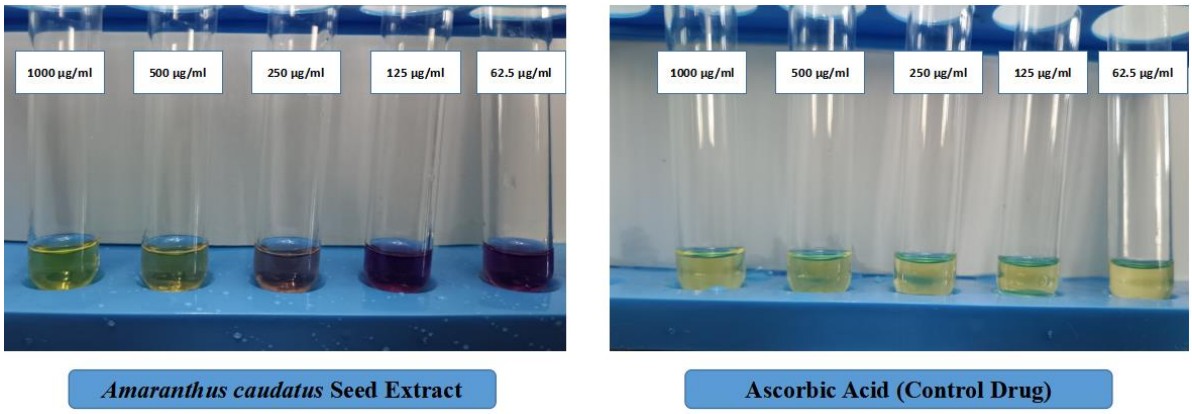

Figure No.4: Anti-Oxidant Activity of *Amaranthus caudatus* seed ethanolic crude extract and Ascorbic acid (Control Drug)

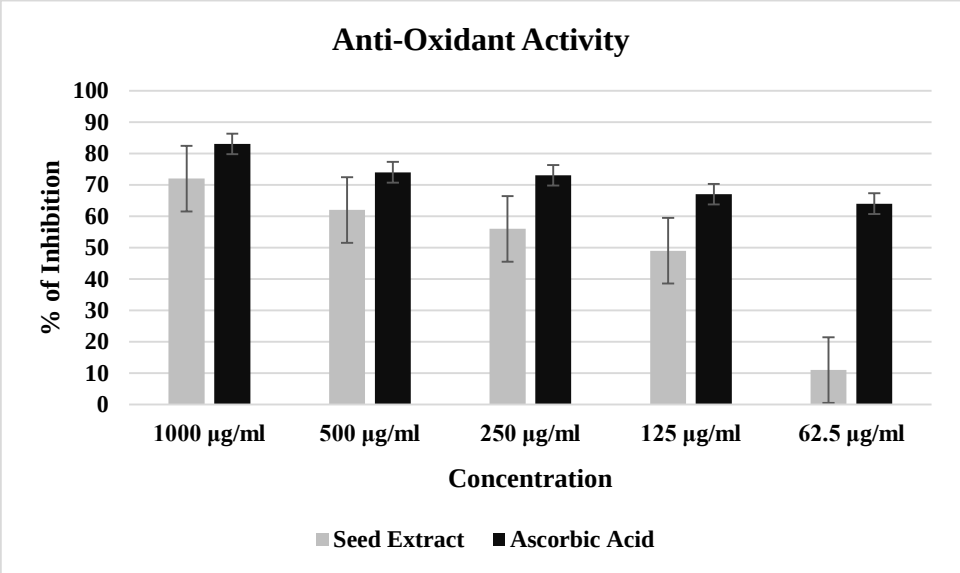

Figure No.5: Anti-Oxidant Activity of *Amaranthus caudatus* seed ethanolic crude extract and Ascorbic Acid

A compound potency is investigated through dose-response studies. EC50 is the centralization of the compound that gives half-maximal reaction. Most of the time, a log-logistic model with EC50 as one of the model parameters is used to evaluate dose-response data. In order to determine a compound's EC50 value, multiple experiments are frequently conducted, necessitating the compilation of EC50 estimates from multiple experiments (Jiang and Kopp-Schneider, 2014). The anti-inflammatory EC50

values of Seed extract was found to be 518.92 µg/ml (Figure 6 and 7) and diclofenac EC50 was observed at 275.19 µg/ml. Likewise, the anti-oxidant EC50 values of Seed extract was found to be 114.25 µg/ml and ascorbic acid EC50 was observed at 288.75 µg/ml (Figure 8 and 9).

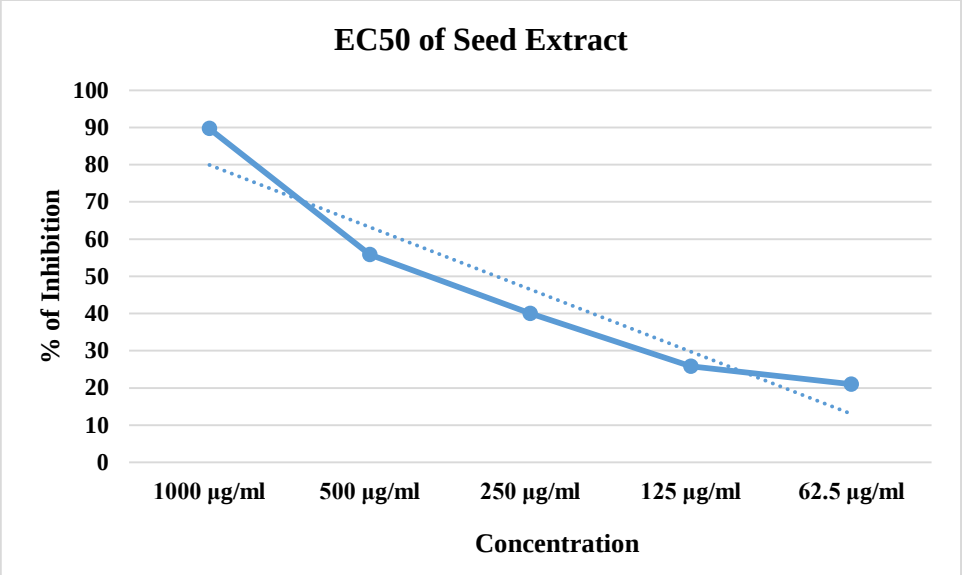

Figure No.6: Anti-inflammatory EC50 plot of Seed extract. The EC50 of Seed extract shown at 518.92 µg/ml.

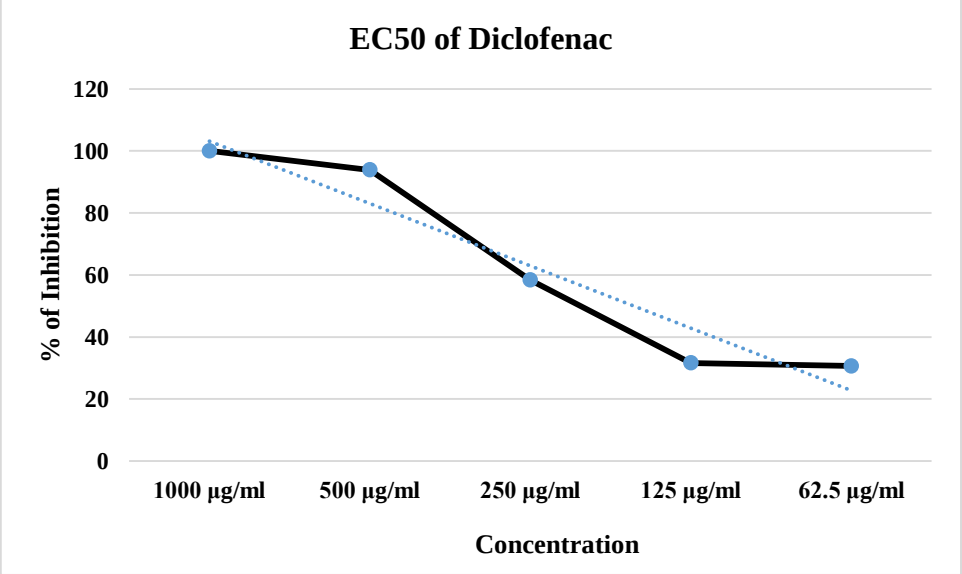

Figure No.7: Anti-inflammatory EC50 plot of Diclofenac Drug. The EC50 of Diclofenac shown at 275.19 µg/ml.

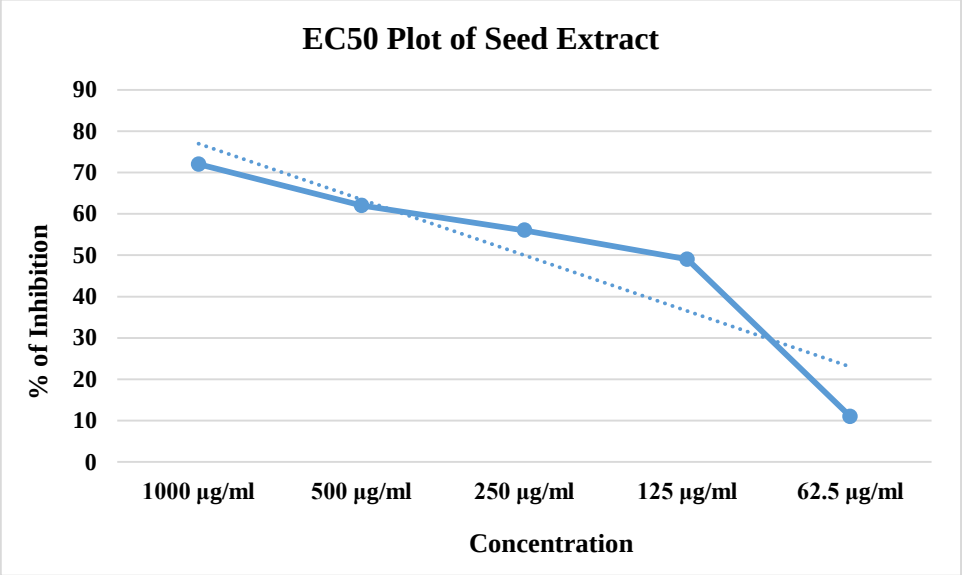

Figure No.8: Anti-Oxidant EC50 plot of Seed extract. The EC50 of Seed Exrct extract shown at 114.25 µg/ml.

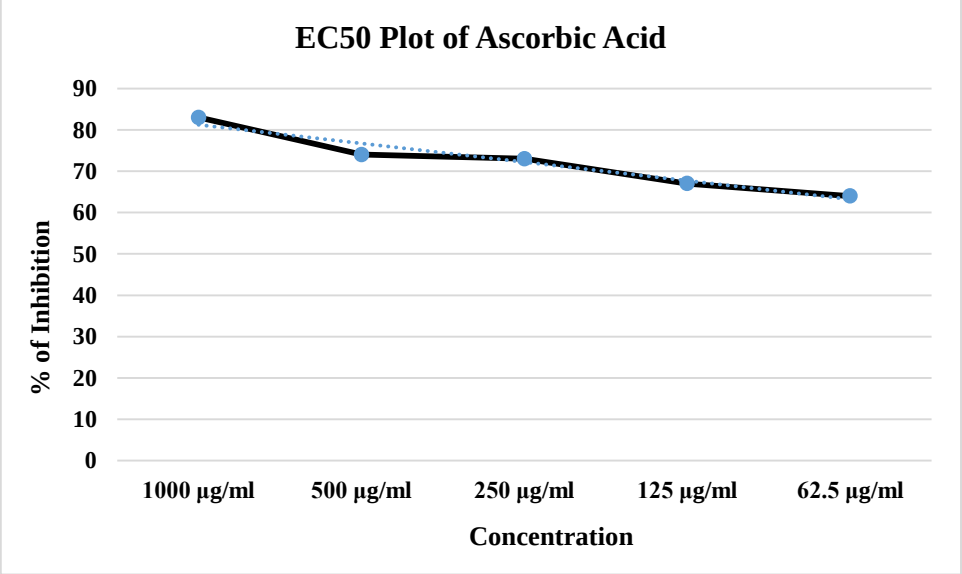

Figure No.9: Anti-Oxidant EC50 plot of Ascorbic acid. The EC50 of Ascorbic acid shown at 288.75 µg/ml.

Discussion

Plants have in without a doubt given the fundamental need of food, cover, clothing, security from sickness causing specialists and therapy of different contaminations and diseases since beginning of mankind's set of experiences. The knowledge of the healing properties of medicinal plants, which are made up of phytochemicals that improve human physiological balance, has been passed down through generations.Many African nations, including Cameroon, Mali, Nigeria, and Zambia, use traditional medicine to meet their health care needs. However, the claims that traditional people make about the usefulness of various medicinal plants in treating diseases have not yet been investigated. As a result, medicinal plants can be the focus of future research (Max et al., 2014; Karou et al., 2011).

Numerous plant antioxidant capabilities are linked to their therapeutic capabilities. The presence of carbohydrates, alkaloids, flavonoids, tannins, and phlobatannins in the extracts of *Amaranthus caudatus*, which may be responsible for the plant's obvious anti-inflammatory and anti-oxidant properties, was found through qualitative phytochemical testing (Akinmalodun et al., 2007; Eleazu et al., 2011). Most of the time, inflammation is the body's response to tissue damage and a number of systemic problems like asthma, atherosclerosis, arthritis, physical injury, and infection (Viljoen et al., 2012). This is consistent with Ahmadiani et al., (2000) report, who claimed that tannins and flavonoids both have anti-inflammatory properties. Numerous enzymes, including phosphodiesterases, phospholipase A2, protein tyrosine kinases, and protein kinase C, can be significantly inhibited by some flavonoids. Numerous enzymes, including phosphodiesterases, phospholipase A2, protein tyrosine kinases, and protein kinase C, can be significantly inhibited by some flavonoids (Middleton E. 1998). Manthey et al., (2001) also reported that some flavonoids inhibit key enzymes involved in the processes of synthesizing prostaglandins. Due to the presence of saponin, tannin, cardiac glycosides, steroids, and alkaloids as well as a higher concentration of triterpenes and flavonoids, ethanol extract had the highest anti-inflammatory potential. This is consistent with the findings of Han and Bakovic (2015), who found that flavonoids inhibit inflammation, and with Hamaleinen et al.,

(2007) research, which found that triterpenoids are biologically active in producing anti-inflammatory effects.

The ability of an ethanolic seed extract antioxidants to convert the purple-colored 1,2-diphenyl 2-picryl hydrazyl (DPPH) radical into the yellow-colored diphenylpicrylhydrazine radical is one of the primary methods for evaluating the radical scavenging activity (Han N, Bakovic M. 2015). A lower EC50 value is associated with a higher radical-scavenging activity of DPPH. Due to its relatively low EC50 values, the ethanol extract had the highest DPPH reducing activity, which was comparable to that of vitamin C (Ascorbic acid) because there was no significant ($p < 0.05$) difference between their EC50 values. The ethanol extract positive results in this test indicate that they contain antioxidants that can neutralize free radicals (Pattanayak et al., 2012).

Conclusion

As a possible future treatment for inflammation, these findings suggest that the ethanolic seed extract of *Amaranthus caudatus* may possess antioxidant properties and provide relief from inflammation. The extract might be used in folk medicine for this reason.

References

1) Ahmadiani, A., Hosseiny, J., Semnanian, S., Javan, M., Saeedi, F., Kamalinejad, M., & Saremi, S. (2000). Antinociceptive and anti-inflammatory effects of Elaeagnus angustifolia fruit extract. Journal of ethnopharmacology, 72(1-2), 287-292.

2) Akinmoladun, A. C., Ibukun, E. O., Afor, E., Akinrinlola, B. L., Onibon, T. R., Akinboboye, A. O., ... & Farombi, E. O. (2007). Chemical constituents and antioxidant activity of Alstonia boonei. African journal of biotechnology, 6(10).

3) Eleazu, C. O., OkaforPN, A. J., Awa, E., & Ikpeama, A. I. &Eleazu KC 2011. Chemical Composition, antioxidant activity, functional properties and inhibitory action of unripe plantain (M. Paradisiacae) flour. African J. Biotech, 10(74), 16948-6952.

4) Evans, W. C. (2009). Trease and Evans' pharmacognosy. Elsevier Health Sciences.

5) Hamalainen M, Nieminen R, Vuorela P, Heinonen M, Moilanen E. (2007). Anti-Inflammatory Effects of Flavonoids: Genistein, Kaempferol, Quercetin, and Daidzein Inhibit STAT-1 and NF-κB Activations,Whereas Flavone, Isorhamnetin, Naringenin, and Pelargonidin Inhibit only NF-κB Activation along with Their Inhibitory Effect on iNOS Expression and NO Production in Activated Macrophages. Mediat Inflamm. 2007:1–10.

6) Han, N., & Bakovic, M. (2015). Biologically active triterpenoids and their cardioprotective and anti-inflammatory effects. J Bioanal Biomed S, 12(005), 1948-5.

7) James, D. B., Kadejo, O. A., Nwochiri, C., & Luca, C. D. (2013). Determination of phytochemical constituents of the aqueous extracts of the leaves, stem bark and root bark of Vitex doniana and its effects on lipid profile of albino rats. British Journal of Pharmacology and Toxicology, 4(6), 210-214.

8) Jiang, X., & Kopp-Schneider, A. (2014). Summarizing EC50 estimates from multiple dose-response experiments: a comparison of a meta-analysis strategy to a mixed-effects model approach. Biometrical journal. Biometrische Zeitschrift, 56(3), 493–512.

9) Karou, S. D., Tchacondo, T., Ilboudo, D. P., & Simpore, J. (2011). Sub-Saharan Rubiaceae: a review of their traditional uses, phytochemistry and biological activities. Pakistan journal of biological sciences: PJBS, 14(3), 149-169.

10) Khalid, M., Alqarni, M. H., Shoaib, A., Arif, M., Foudah, A. I., Afzal, O., Ali, A., Ali, A., Alqahtani, S. S., & Altamimi, A. S. A. (2021). Anti-Arthritic and Anti-Inflammatory Potential of Spondias mangifera Extract Fractions: An In Silico, In Vitro and In Vivo Approach. Plants (Basel, Switzerland), 10(5), 825.

11) Manthey, J. A., Guthrie, N., & Grohmann, K. (2001). Biological properties of citrus flavonoids pertaining to cancer and inflammation. Current medicinal chemistry, 8(2), 135-153.

12) Max, R. A., Mwageni, C., & Bakari, G. G. (2014). Effect of crude root extract from Synadenium glaucescens on selected bacterial infections in albino mice (Mus Musculus). Journal of Medicinal Plants Research, 8(26), 915-923.

13) Middleton Jr, E. (1998). Effect of plant flavonoids on immune and inflammatory cell function. Flavonoids in the living system, 175-182.

14) Viljoen, A., Mncwangi, N., & Vermaak, I. (2012). Anti-inflammatory iridoids of botanical origin. Current medicinal chemistry, 19(14), 2104-2127.

15) Williams, L. A., O'Connar, A., Latore, L., Dennis, O., Ringer, S., Whittaker, J. A., Conrad, J., Vogler, B., Rosner, H., & Kraus, W. (2008). The in vitro anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. The West Indian medical journal, 57(4), 327–331.