doi.org/10.36721/PJPS. 2023.36.2.REG.579-586.1

Physical-chemical analysis of dried *Chamaeleo chamaeleon* powder and evaluation of its aqueous extract antioxidation and antiangiogenesis activity

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Abstract: Our research aims to characterize the physico-chemical properties of *Chameleo chameleon* powder, as well as to evaluate the potency of their compounds as antioxidants in aqueous extracts and their antiangiogenic activity. In this study, physicochemical characterization including pH, ash, some minerals, lipid, protein, carbohydrates and vitamins was investigated. The antioxidant capacity of the aqueous extract of *C. chamaeleon* was determined using DPPH free radical scavenging test and the superoxide radical scavenging test. The antiangiogenic activity was estimated using the chick chorioallantoic membrane assay. The obtained results demonstrate that the chameleon powder has a pH of 5.43 and a dry matter of 90.56%. The mineral richness is demonstrated by the ash result of 22.44% and the quantitative estimation revealed that it includes large levels of phosphorus. A high percentage of proteins, in contrast to carbohydrates and fats, with high values of the examined vitamins were also recorded. According to the results of the antioxidant test, the Chamaeleon extract was very effective in scavenging radicals. In terms of the anti-angiogenesis activity, the extract had very high effectiveness compared to DMSO. These findings lead us to conclude that Chamaeleon is a food supplement had significant antioxidant activity and high activity against angiogenesis.

Keywords: Anti-angiogenesis, antioxidant, *Chamaeleo chamaeleon*, traditional medicine, zootherapy.

INTRODUCTION

Man has been looking for means of survival in nature, such as plants, animals, marine organisms and microorganisms, since prehistoric times, which he employed in the preparation of his food and drink as well as the treatment of numerous illnesses that plagued him. Fossil evidence shows that people have been using plants as medicine for at least 60,000 years (Haidan *et al.,* 2016). The World Health Organization defines traditional medicine as a variety of theories and practices based on the firsthand accounts of many cultures, which are applied to maintain physical or mental health by avoiding or treating diseases (Abdullahi *et al.,* 2011). Humans have employed plants, animals and minerals to create medicines all across the world and have fully assimilated them into modern civilizations, especially phytotherapy and zootherapy (Santos *et al.,* 2019). Zootherapy is the use of animals or animal products to cure a variety of human ailments. It was utilized previously in prehistoric communities and is still used now (Rômulo and Ierecê, 2005).

Several socioeconomic and cultural contexts around the world have documented the usefulness of reptiles in curing and/or preventing illness. The specialized and primarily arboreal reptiles known as chameleons have a variety of derived characteristics. Additionally, they are crucial components in the creation of medications that are therapeutic, protective and preventative for things like disease immunity, protection from witchcraft and bad luck, potency and bringing about good health (Williams and Whiting, 2016).

After being slaughtered and salted, the dried chameleon is known to be employed in the treatment of many illnesses common to the El-Oued region of the Algerian desert. Even local doctors advise their patients to take it because of its proven efficacy, which has been used in some settings, most notably in the treatment of tonsillitis. Additionally, it was applied to the management of thyroid and cough conditions.

The most prevalent variety of chameleon is the Mediterranean chameleon, commonly known as the *Chamaeleo chamaeleon*. It is a kind of oviparous reptile that inhabits hot climates and primarily feeds on insects (Tammy *et al.,* 2006). It goes by a variety of names depending on the region, such as in Oued Souf “El Bouwayh”.

An antioxidant is a chemical that can prevent or significantly slow down the oxidation of errant molecules (free radicals) within a live organism. An oxidizing agent is created during the chemical reaction of oxidation, which can harm various types of cells. Antioxidants stop this series of reactions by eliminating the main ionic intermediate completely and preventing subsequent oxidation processes from oxidizing one another. Antioxidants are defined in a variety of ways, including Chemical compounds that can inhibit oxidation by

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eliminating free radicals, which is one of the processes by which they postpone the commencement of oxidation (Shahidia and Zhongb, 2015).

Angiogenesis is the process of new vessel development and a characteristic of tumor progression (Yadav *et al.,* 2015). For the treatment of various cancers, the development of medications that act as angiogenic inhibitors has become crucial. These antiangiogenic medications' primary goal is to deny tumor cells of oxygen and nutrients, which prevents the creation of new blood vessels and hence slows tumor growth (Ribeiro *et al.,* 2018).

Animal extracts, in contrast to plant extracts, lack the scientific proof to support their biological utility; as a result, the goal of our research was to examine the physicochemical properties of chameleon powder as well as to assess antioxidant and antiangiogenic properties of its aqueous extract.

MATERIALS AND METHODS

Preparation of sample and extraction

The *Chamaeleo chamaeleon* samples (7 animals) were obtained by going chameleon hunting in the Algerian desert's Eloued region. All procedures were done according to the Guidelines for Care and Use of Animals approved by the Ethics Committee of our Institution (Approval No. BCM 022/2019). Samples were then slaughtered, dried at room temperature in the shade and powdered until they had the right consistency. The material was then extracted using a Soxhlet apparatus and distilled water for six hours. The *C. chamaeleon* aqueous extract was concentrated in a rotary evaporator at 45°C. The *extract* obtained was then conserved.

Physicochemical characterization

Using a digital pH meter, the pH of the sample was measured after homogenizing five grams of the sample with 45ml of distilled water in a mixer for one minute and resting for 30 minutes before the measurement. The moisture content was obtained by drying 3g of the sample at 105°C to a constant weight. The ash content was obtained by heating the dry sample to 550°C. The lipid content was determined using the method of Folch *et al*., (1957) and the protein content by the Kjeldahl method (Bennani *et al.,* 1995). The total sugars were extracted according to the method of Martinez *et al.,* (2000) and assayed according to the method of Dubois *et al.* (1956) using phenol and concentrated sulfuric acid. The concentration of iron, zinc, sodium, potassium, calcium, magnesium, phosphorous and copper was determined by an atomic absorption spectrometer, type of JENWAY (PFP7 Flame Photometer) (Longobardi *et al.,* 2012).

Vitamin content

In order to determine the amount of α -tocopherol (Vitamin E), we mixed 1 gram of the sample with 5ml of hexane, homogenized it with a vortex and then centrifuged it for 10 minutes at 5312rpm. After allowing 2ml of the resulting suspension to evaporate at 40°C, we add 2ml of 2-propanol to it. The absorbance was measured at 290nm using the α-Tocopherol standard to create the standard curve (Tchègnon *et al.,* 2017). The results were expressed in mg/100g.

To measure group-B vitamins (B1, B6 and B12), the following steps were adopted: cold 6mL of an aqueous buffer (50% acetonitrile, 1% acetic acid, 0.1% ascorbic acid, 0.1% 2-mercaptoethanol) were used to extract 1g of *C. chamaeleon* powder. *C. chamaeleon* homogenates were incubated for 15 minutes at 50°C in a water bath, quickly chilled on ice and then centrifuged at 2000 g for 15 minutes. The second round of deproteinization was started by adding 2mL of cold acetonitrile (Xu *et al.,* 2020). The clear supernatant was used to determine the absorbance at 280, 260 and 361 nm to asses respectively B1, B6 and B12 (Jin *et al.,* 2012; Chen *et al.,* 2011; Guggisberg *et al.,* 2012). All contents were expressed in mg/100g.

Antioxidant activity

The antioxidant capacity of the aqueous extract of *C. chamaeleon* was determined using the following methods; 2,2-diphenyl 1-picrylhydrazyl (DPPH) free radical scavenging test by spectrophotometer and superoxide radical scavenging test by cyclic voltammetry (Rebiai *et al.,* 2011).

Free radical scavenging test DPPH•

The antioxidant activity of our extract was determined using 2,2-diphenyl 1-picrylhydrazyl free radical scavenging test radicals compared to ascorbic acid using the previously modified method (Talbi *et al.,* 2015). Before analysis, the extract and ascorbic acid at different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7mg/ml) were prepared and mixed volume by volume with DPPH solution (4mg dissolved in 100ml), then incubated for 30 minutes at room temperature in the dark. The absorbance was measured at 517 nm. The antioxidant capacity is determined by the IC_{50} coefficient, calculated by the linear equation of the inhibition ratio change curves, where the percentage of inhibition is estimated with the following relation:

$I\ (\%) = (A_0 - A_S/A_0) \times 100$ (A_0: control absorbance, A_S: extract/standard absorbance).

Superoxide radical scavenging test ($O2•^-$)

The superoxide radical anion is generated by molecular oxygen dissolved in N,N-dimethylformamide (DMF) containing 0.02M of tetrabutylammonium hexafluorophosphate (Bu_4NBF_4) at room temperature (28±1°C). The rotation rate is maintained at 100mV/s. The applicable potential range was -1.6 V to 0.0 V. The studied chameleon extract was added to the superoxide cleavage dissolved in DMF and the voltammograms were recorded

(Rebiai *et al.*, 2011). The ability of the extract to scavenge superoxide radicals (O2˙-) is calculated using the following equation:

$$\%O2^{\cdot-}\ radical\ \text{scavenging activity} = \frac{ipa0 - ipa}{ipa0} \times 100$$

Assay for anti-angiogenesis on chick embryos

Inhibition of blood vessel formation was analyzed by the modified chick chorioallantoic membrane (CAM) assay (Noor *et al.*, 2012). For the first few days of development, eggs are housed in a humidified incubator at a constant humidity and temperature of 37°C. After incubation, sterilized forceps were used to excise a small hole in the surface of the eggshell. To ensure sterility and retain humidity, the formed hole is covered with a sterile laboratory wrap or plastic cover. Before experimenting can start, the eggs are then returned to an incubator that is 50 to 80 percent humidified for a few days. On day 7, the extract was prepared as (50mg/ml), 20µl was deposited on a round disc of Whatman paper and allowed to desiccate before being carried to the CAM and the eggs were resealed and returned to the incubator for 72 hours. On the tenth day, the number of blood vessels was counted. For each treatment group, the vessel branching locations in the square area were analyzed. The mean of the new branching points in each set of samples is used to calculate the angiogenesis score. Each egg received a 1-4 angiogenesis score depending on the number of branching points. The angiogenesis score is 4 if the number of branching points is less than 35. The score is 3 if the branches are between 25 and 34 and 2 if they are 15-24. The score is 1 if the points are 15 (Kamili *et al.*, 2019). All cases were photographed on the 11th day of incubation and appropriate images were taken. The number of blood vessels and their branches, the degree of angiogenesis and the region of inhibition evaluated in the sample around the gelatin sponge are among the variables.

STATISTICAL ANALYSIS

The program *EXCEL* (version 2010) was used to determine vitamin concentrations. The results are provided as mean±standard error after being analyzed with *SPSS-26*. The values obtained from voltammograms analysis were done using *Origin Lab* version 9. The statistical evaluation of the results of antiangiogenesis is carried out by the student T-test; which is based on the comparison between two means. The significance is determined by the value α=0.05, If P<α, there are significant differences between the means and the hypothesis of equality is rejected.

RESULTS

Physicochemical characteristics

The findings of the three-repeat physicochemical analysis of dried *C. chamaeleon* powder revealed that the pH level is 5.43±0.2, which adds to the powder's high storage stability for long periods. We discovered a significant amount of dry matter, 90.56±0.04%, which relates inversely to the amount of water in the dry *C. chamaeleon* powder and also suggests a successful drying process. The *C. chamaeleon* powder's low moisture content (9.43±0.036%) demonstrates the lack of water content in it, extending the powder's shelf life. The findings of burning dry *C. chamaeleon* powder at 550°C revealed that it has a high percentage of ash at 22.44±0.56%, proving that it is a good source of mineral salts necessary for human health.

Chemical composition

According to estimates of the chemical composition of dry chameleon powder, proteins make up a significant portion (38.51%), while the fat content was minimal (5500µg/g) and carbohydrate content was (487.28µg/g). A high percentage of phosphorous, estimated to be 14.01%, was identified in the dry chameleon powder ash and we also discovered considerable amounts of calcium, magnesium, sodium and potassium in proportions of 3.27, 2.83, 1.51 and 0.49%, respectively. The results of zinc, iron and copper indicated that these elements were present in extremely trace amounts in the chameleon powder.

Table 1: Chemical composition of *Chamaeleo chamaeleon*

Parameters	Moy ± δ
Protein (%)	38.51±7.29
Total lipid (µg/g)	5500±34
Total sugars (µg/g)	487.28±4.64
Phosphorus PO_4 (%)	14.01±0.11
Calcium Ca (%)	3.27±0.80
Magnesium Mg (%)	2.83±0.04
Sodium Na (%)	1.51±0.077
Potassium K (%)	0.49±0.04
Copper Cu (%)	0.003±0
Zinc Zn (%)	0.003±0.00057
Iron Fe (%)	0.002±0.00057

Vitamins content

The results of assessing the vitamin content reveal that the chameleon is a rich source of vitamins, as indicated in the table below, where we discovered high levels of vitamin E and group B vitamins (B1, B6 and B12).

Table 2: Contents of group B vitamins and α-tocopherol in *Chamaeleo chamaeleon.*

Parameters	Moy ± δ
α-tocopherol (mg/100g)	19.23±0.30
Vitamin B1 (mg/100g)	1.21±0.06
Vitamin B6 (mg/100g)	3.60±1.51
Vitamin B12 (µg/100g)	5.61±0.30

Antioxidant activity

The DPPH radical inhibition percentage was measured to assay the antiradical activity of the extract. The least DPPH radical scavenging activity was detected in the aqueous

extract of dried *C. chamaeleon* with an IC_{50}= 0.45±0.08mg/ml, compared with Ascorbic Acid IC_{50}= 1.29±0.41µg/ml.

Superoxide radical scavenging test (O2˙−)

The reactive oxygen free radicals were produced after obtaining the previously determined experimental conditions at room temperature, the form (A) in fig. 1 identifies the voltammetric parameters of oxygen in the absence and the presence of the aqueous extract of dried *C. chamaeleon*. From the values of the voltammetric parameters, the regression line of the change in the percentage % of O_2˙- inhibition according to the concentrations of the aqueous extract can be obtained, form (B) fig. 1.

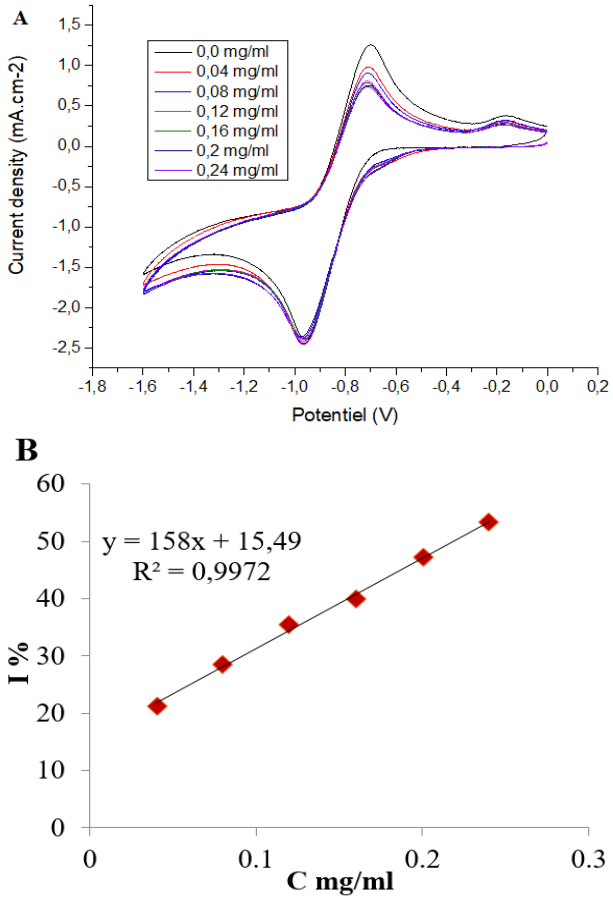

Fig. 1: the rate of inhibition of superoxide radical anion (O2˙-) for the aqueous extract of *C. Chamaeleon*. (A) Cyclic voltammograms of (O2˙-) radical in the absence and presence of increasing concentrations of the extract were recorded on a glassy carbon electrode at a potential sweep rate of 100 mV/s. (B) Regression line of Inhibition (I%) according to the concentrations of the extract (C mg/ml).

The results obtained show that the extract has the highest IC_{50} value of 0.22mg/ml, indicating that it has good antioxidant activity in superoxide radical scavenging when compared to α-tocopherol, which has an IC_{50} of 3.04mg/ml.

Anti-angiogenesis activity

The ability to suppress CAM angiogenesis on day 11 for the *C. chamaeleon* aqueous extract as compared to positive and negative control was studied. Angiogenesis was reduced in the samples treated with AQ, as demonstrated in fig. 2 and table 3. Blood vessels are indicated by arrows in the photos. The squares encircle the new branches that accompany them. Many blood vessels and, naturally, a considerable number of branches formed in the DMSO-treated group. A tiny number of blood vessels show in the picture of the group treated with Bevacizumab, together with the absence of new branches. The findings of the AQ group were similar to those of the Bevacizumab group, with fewer vessels.

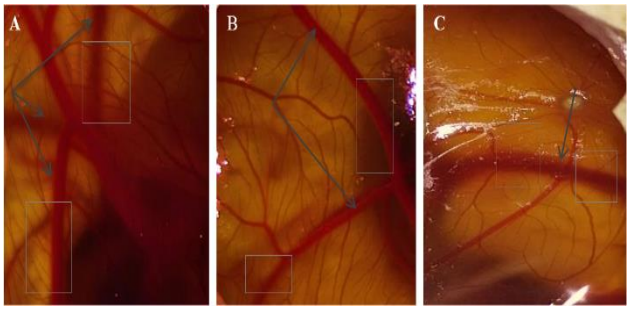

Fig. 2: CAM assay images of control and treated eggs with aqueous *C. Chamaeleon* extract. (A): negative control (DMSO), (B): positive control (Bevacizumab), (C): aqueous extract (AQ).

When compared to the control group, the number of blood vessels and branching points in the group treated with chameleon aqueous extract showed a significant difference, but not when compared to the Bevacizumab group. Furthermore, chameleon extract, like Bevacizumab, reduce significantly ($p<0.01$) the angiogenic when compared to the DMSO group. The results showed also that the zones of inhibition recorded with *C. Chamaeleon* aqueous extract and Bevacizumab were significantly higher than the control group.

Table 3: Average number of vessels, branching points, number of angiogenesis score and inhibition zone in the samples treated with aqueous extract of *C. chamaeleon*.

	DMSO	Bevacizumab	AQ
No. of vessels	12.20±3.27	4.40±1.14	$3.00±1.41^{**,NS}$
No. of branching points	22.40±3.58	13.00±1.22	$14.00±3.39^{*,NS}$
Angiogenesis Score	2.40±0.55	1.00±0,00	$1.60±0.05^{**,NS}$
Inhibition Zone (mm)	8.80±1.30	19.40±2.19	$20.00±3.61^{**,NS}$

(AQ) compared with DMSO: *p<0.05 and **p<0.01
(AQ) compared with Bevacizumab (NS: not significant).

DISCUSSION

The common chameleon is a type of reptile that was once used to treat a variety of illnesses in the Oued Souf region. Following the locals' traditional method of preparation, the studied sample was prepared by being slaughtered and dried for at least a week. Next, by conventional use, we extracted it using water as a solvent. The pH of chameleon powder was measured as part of the physicochemical analysis since a lower pH impacts the quality of meat preservation and lengthens the meat's shelf life during storage and handling because the bacteria that destroy meat grow more slowly as the pH drops (Rahman *et al.,* 2005). The chameleon powder's pH level was as low as 5.43, which lengthens its shelf life, according to our research. Additionally, the outcomes are comparable to those of (Menéndez *et al.,* 2018), where 5.35 were discovered for horse cecina and 5.24 for the cow-cured tongue. The results of the determination of moisture 9.43% and dry matter 90.56% demonstrate the effectiveness of the chameleon drying process and its potential for longer shelf life. According to Toumi-Nesri (2018), the water content shouldn't exceed a value between 10 and 12 if we want to avoid bacterial change. When the moisture rate exceeds 14, the conditions are favorable for the formation of mold. The results obtained are close to what was found by Rahman *et al.,* (2005) with a value of dry matter equal to 94 for air-dried meat.

Finding the sample's ash percentage, which equals 22.44%, reveals that the chameleon powder has a high metal content; this number is caused by the bones and minerals present in the skin (Toumi-Nesri, 2018). Where we discovered the highest concentration of phosphorous PO_4 (14.01%), we then discovered the highest and most significant calcium concentration (3.27%). Additionally, magnesium represents a significant fraction, which is 2.83%. The outcomes are comparable to those observed in fish (Jim *et al.,* 2017). In comparison to chameleon meat, young goat meat contains a larger percentage of calcium and magnesium (Longobardi *et al.,* 2012). Additionally, the ratio of potassium 0.49% and sodium 1.51% is not taken into account. Unlike what was observed by Longobardi *et al.,* (2012) in the meat of young goats and comparison to the Sandfish (Toumi *et al.,* 2017) and the meat of young goats (Longobardi *et al.,* 2012), the proportions of zinc (0.003%, copper 0.003% and iron 0.002% are weak.

The sample's protein content was found to be 38.51%, which is typical for animal sources. These findings are comparable to Toumi *et al.,* (2017) data for Sandfish flour (46.28%) and superior to those found by Longobardi *et al.,* (2012) in young goats meat (18.9%) and by Malti and Amarouch (2008) in naturally fermented camel meat sausage 19.13%. Additionally, a key determinant of nutritional value is protein content. The findings lead us to believe that chameleons are an essential source of protein. The results of the fat content of chameleon powder demonstrate that there is a small amount of fat equal to 5500±34µg/g, which enhances the quality and validity of its nutritional value. The very high fat content harms the final quality and harms preservation by exposing the meat to early rancidity (composition peroxide) (Hosseindoust *et al.,* 2020). As is the case with all animal sources, the sugar content of chameleon powder is minus and equal to 487.28±4.64µg/g and meat has low sugar content because glycogen converts to lactic acid after an animal dies (Toumi-Nesri, 2018).

Along with proteins, carbohydrates and fats contents, we also looked at the amount of α-tocopherol 19.23±0.30 mg/100g and vitamins B1 (1.21±0.06mg/100g), B6 (3.60±1.51mg/100g) and B12 (5.61±0.30µg/100g) to assess the nutritional value. This proved and increased the chamaeleon powder's significant therapeutic value. Vitamin E is important for vision, reproduction and the health of the blood, brain and skin. It also contains antioxidant properties (Dattola *et al.,* 2020; Nasimi *et al.,* 2021). Moreover, the body needs thiamine to maintain the health of the liver, eyes and nails and vitamin B1 plays a key part in metabolic processes and affects the trend for effective antioxidant activity (Piechocka *et al*., 2021).

Additionally, when comparing vitamin B1 content in chameleon powder, our results were higher than what was discovered in pork, which had a value of 0.72±0.18 mg/100g (Esteve *et al.,* 2002).

Regarding vitamin B6, it is essential to strengthening the immune system because it helps to produce lymphocytes and antibodies and regulates blood pressure and cholesterol levels. Regarding vitamin B6, it is crucial to fortify the immune system because it contributes to the production of lymphocytes and antibodies and controls cholesterol and blood pressure levels (Mamede *et al.,* 2011).

Vitamin B12 contributes to building body cells, strengthening nerves and contributing to the prevention of skin cancer (Abourazzak *et al.,* 2012). When comparing the results of B12 with meat, the chameleon powder is very rich than dried beef, which contains 1.12-2.61µg/100g according to Gille and Schmid (2015).

The results of the antioxidant activity of the AQ showed that it is effective in scavenging radicals (DPPH$^{\bullet}$ and superoxide). In terms of the anti-angiogenesis activity, the extract had very high effectiveness through significant differences in vessel numbers, branching points, angiogenesis score and inhibition zone compared to DMSO.

The antioxidant and anti-angiogenic efficacy of *C. Chamaeleon* extract may be attributed to the constituents present in the extract.

The antioxidant potency of the extract may be attributed to the presence of vitamins, like the water-soluble vitamin B6 and B12, known for their antioxidant capacity (Birch *et al.*, 2009; Giustina *et al.*, 2019), free amino acids or small peptides; fatty acids. Moreover, according to Boudebia *et al*. (2022), the infrared analyses of the chameleon aqueous extract revealed the existence of several functional groups that can interfere with the different biological activities of this extract.

It has been shown that a variety of substances, including peptides, proteins, pharmacological agents, medications, metabolites, plant extracts, biomaterials and nanoparticles, can suppress angiogenesis in CAM assay (Kennedy *et al*. 2022).

An organized series of processes called angiogenesis is governed by a number of pro- and anti-angiogenic growth factors. The anti-angiogenic activity of our extract may be the result of the interaction of components present in the extract with these angiogenic growth factors.

According to Ardura *et al.*, (2020), calcium**,** one of the minerals found in the AQ, can help to prevent the creation of vessels Tumor cell death, proliferation, motility-invasion and tumor metastasis are all affected by calcium. Calcium regulates the molecular components and signaling pathways involved in the genesis and progression of prostate cancer. Calcium channels and calcium-binding proteins are examples of such factors and processes. According to Thundimadathil (2012), there is a huge effort to find angiogenesis inhibitors based on peptides as the safest and least harmful treatment for disorders linked with aberrant angiogenesis. Vitamin B6 is one of the vitamins that have a key function in the prevention of tumor angiogenesis. Also, several types of research have shown that vitamin B6 has bioactivity as a cell growth inhibitor *in vitro* cancer models (Peterson *et al.*, 2020).

The findings of this study lead us to suggest that *C. Chamaeleon* is a food supplement that is high in minerals and proteins and has tremendous beneficial effects on health. However, the use of extract by humans was limited for inconclusive scientific evidence to support their use as a treatment for specific disorders. Furthermore, the extract may have potential side effects or interact with other medications, making it unsafe for some individuals to use.

CONCLUSION

Natural sources of animal origin require much research because they contain so many valuable components. In our current study, we discovered that dry *C. Chamaeleon* powder has a high nutritional and therapeutic value since it contains a significant amount of proteins and numerous beneficial minerals like phosphorus, calcium, magnesium, sodium and potassium. Additionally, there are vitamins such as vitamin E and group B vitamins B1, B6 and B12. As a result, the dry *C. chamaeleon* powder's aqueous extract had significant antioxidant activity and high activity against angiogenesis, making it a strong candidate for the prevention of tumor growth and the treatment of cancer. Considering the importance and novelty of this subject, it would be intriguing to pursue further research into the characterization of protein amino acids and the identification of additional vitamin types and to continue the search for precise results using additional solvent types and clinical studies.

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