Antioxidant Properties and Anti-Inflammatory Activities of *Cucumeropsis Edulis* (Seeds)

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Research Article

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Abstract

This study sought to explore the medicinal properties of extracts obtained from *C. edulis* seeds. The seeds were obtained from farms in the Upper East Region of Ghana, dried and milled into coarse powder. Petroleum ether, ethyl acetate and methanol were used to extract the bioactive compounds present in the seeds by the cold maceration method. Antioxidant properties of the extracts were evaluated using the Phosphomolybdenum and DPPH free radical scavenging assays. The Folin-Ciocalteu assay was used to estimate the total phenol content and carrageenan-induced paw oedema model in chicks employed for the anti-inflammatory effects. The results showed that methanol extract had the highest antioxidant and anti-inflammatory activities while the petroleum ether extract showed the least activity. The anti-inflammatory activity of the methanol extract (31.3% oedema inhibition at 300 mg/kg body weight) was however lower than diclofenac (54.04% oedema inhibition at 100 mg/kg body weight), the reference drug. Compounds such as tannins, alkaloids, and carotenoids detected during phytochemical screening may be responsible for the activities observed.

1.0 Introduction

Antioxidants are chemical species with the ability to postpone oxidation or inhibit the propagation stage of free radical reactions in living cells. In the living cell, the destruction of cellular macromolecules such as proteins, carbohydrates, lipids and nucleic acids by free radicals causes degenerative diseases including cancer, cardiovascular disease, cataracts, Alzheimer's disease, atherosclerosis, hypertension, diabetes mellitus and aging (1). There has been an increased interest in the study of antioxidant compounds because their presence has been shown to be efficacious in inhibiting free radicals hence slowing or quenching the emergence of degenerative diseases. Reports in literature indicate that antioxidants improve human health by boosting the immune system, inhibiting cancer cells, reducing cardiovascular diseases and diabetes (2) effect healing of chronic ulcers (3). They have also been shown to possess anti-allergenic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio-protective and vasodilatory effects (4, 5).

Naturally occurring antioxidants are present in different forms, they include compounds such as phenolics, flavonoids, coumarins, xanthones, lignans, tannins, curcuminoids, tocopherol, lycopene and β-carotene (5). They can be found in the fruits, leaves, seeds, and oils of plants (6).

Edible oilseeds are reported to be good sources of compounds with biological activity (7). Such seeds produce diverse kinds of secondary metabolites with a wide range long-term therapeutic and physiological potential (8). The oilseeds may contain secondary metabolites with the ability to induce carcinogen detoxification, prevent tumor growth and block carcinogens, as well as exhibit antioxidant properties which can improve overall immune response (9, 10). Supplementing the body with exogenous antioxidants or boosting the antioxidants in the body is reportedly beneficial in fighting the undesirable and unwanted effects of oxidative damage induced by Reactive Oxygen Species (ROS). Synthetic antioxidants have been commonly used as food additives to help manage or minimize the risk of certain

cancers, cardiovascular diseases, coronary heart diseases and in some cases ageing (11, 12). Epidemiological studies have shown that the inclusion and intake of antioxidant substances in our diets reduces death ascribed to chronic degenerative diseases (12).

Cucumeropsis edulis seeds (Melon seeds) are an example of such oilseeds found in abundance in the savannah zones of Ghana. *Cucumeropsis edulis* (*C. edulis*), commonly known as melon and locally called “wrewre” in Ghana. It grows in rich sandy soils in the hot climate zones of Africa. Dried seeds of *C. edulis* are usually roasted, milled, and used as sauce thickener (13) as well as making soups and stews in some parts of Ghana and neighboring countries including Nigeria and Burkina Faso. The *C. edulis* seeds have been reported to have excellent nutritional value (14). It is rich in carotene (Provitamin A), essential amino acids, vitamins, and mineral elements (15, 16).

In the current study, antioxidant properties and the anti-inflammatory activities the *C. edulis* seeds were studied. The bioactive compounds in the seed were extracted with solvents of different polarities. The interest is to study the antioxidant and the anti-inflammatory activities of the extracts obtained from different solvent types. Polyphenols are the most abundant group of phytochemicals, different kinds of polar solvents for example; methanol, ethanol and acetone have been employed in their extraction from the plant source (17).

The aim of this study is to investigate medicinal activities of *C. edulis* seeds. Knowledge of the nature of the secondary metabolites and their solubility in a particular type of solvent (polar or nonpolar) will significantly inform the method of processing food products from the seeds of *C. edulis* for enhanced medicinal benefits.

2.0 Materials And Methods

2.1 Plant material harvesting and preparation

The seeds for the study were obtained from farms in the Upper East region, Ghana, All plant sample were collected and prepared in accordance with the Kwame Nkrumah University of Science and Technology (KNUST), Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences guidelines and regulations in the use of plant samples. By this, where the plant is endmeic. Permission to harvest the seeds was sought from the indigenous farm owners in the region. The seeds were identified by a botanist, Mr. Clifford Asare, of the herbarium section of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, where a voucher specimen has been deposited (**KNUST**/HM1/2018/S05). The seeds were cleared of any extraneous matter by sorting and sieving. The clean seeds were air-dried, powdered, and securely stored for future purposes.

2.2 Reagents and chemicals

Gallic acid, ascorbic acid, Folin-Ciocalteu (FC) reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were procured from Sigma-Aldrich, UK (United Kingdom). Analytically pure reagents and drugs were used for

this research.

2.3 Extraction of plant sample

The powdered seeds (500 g) were extracted serially with petroleum ether first, followed by ethyl acetate and finally methanol. Each extraction period lasted 72 hrs to ensure exhaustive extraction. The extracts were taken through a filtration process, and the filtrates concentrated in a rotary evaporator (R-114, Buchi, Switzerland) at 60°C temperature and reduced vacuum pressure. The concentrates were dried on a water bath by evaporation and kept in a desiccator for further analysis. The extracts were coded PEE: petroleum ether extract, EAE: ethyl acetate extract and ME: methanol extract.

2.4 Antioxidant activity assays (In-vitro)

2.4.1 DPPH free radical scavenging assay

The potential of the extracts in comparison to ascorbic acid, the reference compound, to scavenge free radicals was done employing a method described by (18). In brief, DPPH methanol solution (3 mL, 20 mg/L) was added to 1 mL each of extract (1000 – 31.25 µg/mL) in test tubes. The reaction mixture was kept in the dark for incubation lasting 30 minutes at room temperature. The same process was done for different concentrations (200- 3.125 µg/mL) of ascorbic acid. Absorbance of the residual DPPH in reaction mixture was determined in a UV-visible spectrophotometer at 517 nm. The radical scavenging activity was estimated from the equation stated below:

$$\% \text{ DPPH radical scavenging activity} = 1 - \left(\frac{\text{Absofsample}}{\text{Absofcontrol}}\right) \times 100\%$$

The percentage DPPH free radical scavenging activity was then plotted against log of the concentration of the standard and extracts. The concentration of the extracts and standard needed to scavenge 50 percent of DPPH radicals was expressed as IC□□.

2.4.1 Total antioxidant activity (Phosphomolybdenum assay)

The phosphomolybdenum method is based on molybdenum (Mo□□) reduction by extracts and subsequently forming green phosphate-molybdate (Mo□□) complex in low pH environment. Disodium hydrogen phosphate (28 mM), ammonium molybdate (4 mM), and sulphuric acid (6 mM) were prepared and added to make the reagent solution. Solutions of the different extracts were also prepared by dissolving the extracts (0.05 g) in distilled water and diluted to make up 500 µg/mL. To 1 mL of the prepared extract solutions, the reagent solution (3 mL) was added and at 95°C, it was incubated for 90 mins. The ascorbic acid solutions (100-0.781 µg/mL) were prepared. 1 mL of the solutions were taken, the reagent solution (3 mL) added and at 95°C incubated for 90 mins. For absorbance measurements, UV- Vis spectrophotometry was used at 695 nm to plot the standard curve. The total antioxidant activity

was calculated from the equation of the line obtained from the standard curve and expressed in mg per g ascorbic acid equivalent of the extract (AAE) (18).

2.5 Analysis of total phenol content

Spectrophotometrically, the total phenol content was estimated employing a method described by (19) with slight modifications. Gallic acid solution (100-3.125µg/mL) was used as a reference compound for plotting the standard curve by mixing 0.5 mL of the extract with Folin-Ciocalteu (2.5 mL, 10%) and neutralized with aq. Na□CO□ (2 mL: 75 mg/mL) in test tubes. The reaction mixture in an oven was incubated for 10 mins at 50°C. The absorbances were measured at 760 nm using UV-Vis spectrophotometry. To 0.5 mL of the different extracts, 0.05 g was dissolved in water to make up a concentration of 500 µg/mL, 2.5 mL 10% FC reagent was added and neutralized with 2 mL aq. Na□CO□. The reaction mixture was incubated for 10 mins at 50°C and absorbances measured at the same wavelength. The equation of the line obtained from the calibration curve was used for the total phenol estimation and the results reported in equivalents of gallic acid (mg GAE/ g extract).

2.6 Anti-inflammatory activity (In- vivo)

2.6.1 Test Animals

Chicks (Gallus gallus), one-day post-hatch, were procured from the Department of Animal science poultry farms under the Faculty of Agriculture and Natural Resources, KNUST, Ghana. The chicks were kept in cages made of stainless steel (with dimensions 34x57x40 cm^3) at population density of 12–13 chicks per cage, and the room temperature maintained at (29 ± 10°C). Feed (Chick Mash, GAFCO) and water were provided. An elevated incandescent lighting was kept over the chicks on a half-day light-dark pattern. Routine care was given to the chicks until the seventh day when the experiment was conducted. The experimental protocol was carried out in accordance with guidelines set by the Institute for Laboratory Animal Research (Organisation for Economic Co-operation and Development (OECD), 2001) for care and use of experimental animals and approved by the Department of Pharmacology's Ethics Committee (PCOL/ETH/230001), KNUST. The experiment is reported herein as detailed by the Animal Research Reporting of in vivo experiments (ARRIVE) guidelines (ARRIVE.org).

2.6.2 Anti-inflammatory activity

Chicks were weighed on the seventh day and divided randomly into control and treatment groups to be used for evaluating the anti-inflammatory activity of the extracts. Carrageenan-induced paw oedema model described by (18) with modifications was used. Group sizes of five were used for the experiment. Time zero reading of the chicks' foot volume was measured using a caliper before inducing the footpad oedema by sub-plantar injection of carrageenan solution (10 µL :1% w/v saline) into the right foot pads. The foot volumes were measured 1hr after injection and the extracts; PEE, EAE and ME orally dosed at 300, 100 and 30 mg/kg body weight of the chick. Diclofenac, a standard drug for treating inflammation, (10, 30 and 100 mg per kg) was given orally and the control chicks received saline only. Hourly readings of the foot volumes were taken for 5 hrs. The oedema was evaluated by measuring the foot volume

differences before the injection of the carrageenan and at the different time intervals. The percentage inhibitions of the oedema component of the inflammation for the treated groups were determined using the equation:

$$\% \text{ oedema inhibition} = \left(\frac{AUCcontrol - AUCtreatment}{AUCcontrol} \right) x\ 100\%$$

2.7 Phytochemical screening of powdered plant sample

The powdered seeds were taken in batches and screened for the presence of selected phytoconstituents using standard assays (20, 21).

2.8 Statistical evaluations

All results of the study were recorded as mean ± standard deviation. Both statistical and graphical data analysis was done with GraphPad prism software (sixth version). The differences in AUCs (Area under curves) were analyzed with one-way analysis of variance (ANOVA) and Newman-Keuls post hoc test after. $P < 0.05$ signified the results statistically significant and different.

3.0 Results

3.1 Antioxidant activity of melon seeds

The antioxidant potential of extracts from *C. edulis* were evaluated using the DPPH free radical scavenging assay, the total antioxidant capacity (TAC) assay and the total phenolic content (TPC) assay. Results from the DPPH free radical scavenging assay is shown in Fig. 1. The results show that the free radical scavenging activity increases as the concentration of extracts increases. The extent of free radical scavenging activity of the extracts was estimated using the IC□□. From the results, it was revealed that differences in polarities of the solvents had a significant effect on the scavenging potential of the extracts with an order, Methanol extract □ Ethyl acetate extract □ Petroleum ether extract (Table 1). Data recorded shows that the free radical scavenging activities were significantly different ($p < 0.05$).

Table 1
The DPPH scavenging activities of the extracts and reference compound.

Extract/Drug	PEE	EAE	ME	Ascorbic acid
IC_{50} (µg/mL)	513.9	292.8	184.5	11.63

PEE (Petroleum ether extract); EAE (Ethyl acetate extract); ME (Methanol extract)

The phosphomolybdenum assay was employed to assess the TAC of the extracts from *C. edulis* seed. The total antioxidant capacity from the assay was evaluated and quantified based on their ability to

reduce Mo□□to Mo□□. The methanol extract exhibited the highest total antioxidant capacity of 448.0 mg/g AAE, followed by the ethyl acetate extract and lastly, the petroleum ether extract (Table 2).

Additionally, the TPC of the extracts was quantified based on the ability of inherent phenolic compounds to transfer electrons in alkaline medium to form a blue colored phosphotungstic/phosphomolybdenum complex with the Folin-Ciocalteu reagent (22, 23). The results obtained from all three extracts were statistically different with p-value < 0.05. It was revealed again, that the methanol extract gave the highest total phenol content (78.01 mg/g GAE) and petroleum ether extract the least (13.55 mg/g GAE).

Table 2
Total phenolic content and total antioxidant capacity of the extracts

Extracts	TPC (mg/g GAE)	TAC (mg/g AAE)
PEE	13.55 ± 0.413	388.4 ± 2.778
EAE	42.48 ± 4.294	423.1 ± 9.260
ME	78.01 ± 1.040	448.0 ± 14.82
Note: All data were recorded as mean and their standard deviation. PEE (Petroleum ether extract); EAE (Ethyl acetate extract); ME (Methanol extract)		

To understand the relationship between TPC on TAC, a comparative study of the Total Phenol Content and Antioxidant Capacity of the extracts was conducted, and the results (Fig. 2) revealed a positive correlation (i.e. TAC increases with increasing TPC). To evaluate the significance of the phenolic content on the antioxidant capacity, the coefficient of distribution and correlation coefficient between the two parameters were calculated for each extract (Table 3). Thus, about 75.23% of the antioxidant property with regard to the methanol extract was due to its phenolic content, whilst the other metabolites make up the remaining 24.77%

Table 3
The correlation coefficient (r), and coefficient of distribution (r^2) between TPC and TAC of the extracts.

Extracts	Pearson correlation coefficient (r)	Coefficient of distribution (r^2)
PEE	0.8161	0.6660
EAE	0.8272	0.6843
ME	0.8673	0.7523

3.2 Anti-inflammatory activity

The anti-inflammatory assay used employed a curative approach that investigates the ability of the extracts to reduce inflammation. The results showed that the different doses, 30–300 mg/kg body weight for the extracts and 30–100 mg/kg body weight for diclofenac (the reference drug), dose-dependently inhibited the foot oedema induced in the chicks to varying degrees (Figs. 3 and 4). Again, the methanol extract exhibited a significant anti-inflammatory activity with a maximal percentage inhibition of 31.32% compared to ethyl acetate and petroleum ether extracts respectively (Fig. 3a, b, c, d and 4a, b).

4.0 Discussion

The presence of secondary metabolites in plant-based diets has been reported to be responsible for the prevention many diseases and hence promoting good health (10, 24). These secondary metabolites may act as natural antioxidants (scavenge free radicals in the body) to reduce the risk and progression of certain ailments including cancers, stroke, cardiovascular and autoimmune diseases. (25–28).

Natural products with an antioxidant property have the ability to quench reactive free radicals and prevent oxidation of other molecules. In this study, the antioxidant activity of the extracts were evaluated using the DPPH free radical scavenging activity assay. The results revealed a concentration dependent on the scavenging activity for all extracts as well as the standard ascorbic acid. Evaluation of the activity of the extracts on radical scavenging was conducted using the IC□□. It was observed that the methanol extract gave a high potency even at low concentration. The IC□□ represents the concentration (µg/mL) of compound/ plant extracts needed to mop up half the free radicals in a system. This implies that, from the study the methanol extract has a high ability to mop up about 50 % of free radicals in the system even at lower concentrations. Secondary metabolites found in plants, especially phenolic compounds are known to have significant health benefits as a result of their scavenging action. The presence of phenolic compounds has been shown to play vital roles in antioxidant and anti-inflammatory potentials of natural products (29). Phenolic compounds are essential compounds in diet and medicine due to their ability to mop up free radicals, superoxide, and hydroxyl radicals (30). The antioxidant potential of phenols has been reported to be as a result their redox property, which gives them the ability to behave as hydrogen donors, reducing agents, singlet oxygen quenchers (25, 31–32) and metal chelating potential.

In this study, the phenolic content of the three extracts followed the trend of methanol extract > ethyl acetate extract > pet-ether extract. Many studies have shown that, the presence of phenolic compounds in plant-based foods has significant effect on the antioxidant properties of such foods (17). Again, research has shown that most phenolic compounds are soluble in methanol, ethanol, hot water, acetone and ethyl acetate (33). This implies that the high that the phenolic content of the methanol extract is in agreement with literature. According to reported data, Li and co-workers in their study “Total phenolic contents and antioxidant capacities of 56 selected Chinese medicinal plants” revealed a range of 0.12 to 59.43 mg GAE/g of phenolic content (34). Again, the total phenolic content found in six important fruit residues as reported by Oberoi et al range from 3.68 to 37.4 mg GAE/g (35). Therefore, considering a phenolic content of 78.01 mg GAE/g from the methanolic extract as recorded in this study implies *C. edulis* seeds can be classified as a good source of phenolic compounds.

The high antioxidant capacity recorded for the methanolic extract which recorded the highest phenolic extract also confirms the effect of the phenolic compounds on the antioxidant potential of the extracts. Further study on the relationship between the total antioxidant capacities and total phenolic contents gave a linear positive relationship. The strong correlations between the total antioxidant capacity and the total phenolic content showed that phenol compounds largely contribute to the antioxidant activity of the seeds, and therefore could play an important role in the beneficial effects of these plants. The results were in accordance with other research works (36, 37). To evaluate the significance of the phenolic content on the antioxidant capacity, the calculated coefficient of distribution and correlation coefficient between the TAC and TPC which revealed that about 75.23% of the antioxidant property of the methanol extract might be due to its phenolic content. Therefore, the TAC of a particular extract may not only be attributed to its phenolic content but the presence of other phytochemicals.

The antioxidant potential of phenolic compounds and other phytochemicals exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory activities. Inflammation is associated with virtually all diseases and there has been intense research to obtain new anti-inflammatory agents as alternative to conventional ones whose use is limited by a plethora of side effects. Inflammation is known to be related to oxidative stress, a condition resulting from excess free radicals in the body and associated with almost all diseases hence the need for natural anti-inflammatory agents (38) which could be in the form of diets or drugs (37, 39). During inflammation, the release of ROS by phagocytic leukocytes- if unregulated, may overwhelm the body's antioxidant system and result in chronic inflammation. Thus, the antioxidant activities shown by the extracts of *C. edulis* will be notably helpful in ameliorating the impact of ROS in the body and also during the inflammatory cascade. This was confirmed by the high anti-inflammatory activity of the extracts especially that of the methanolic extract.

5.0 Conclusion

The present study has shown that *C. edulis* seeds contain essential bioactive compounds with considerable antioxidant, anti-inflammatory activities and high phenolic content. Consumption of the seeds can contribute to maintaining good nutrition and health. Preparation of melon seeds with polar solvents such as water may offer the best health benefits.

Declarations

AVAILABILITY OF DATA

All generated and analyzed data in this research work are incorporated in the preparation of this manuscript and upon request will be made available from the corresponding author.

CONFLICT OF INTEREST

FUNDING STATEMENT

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Figures

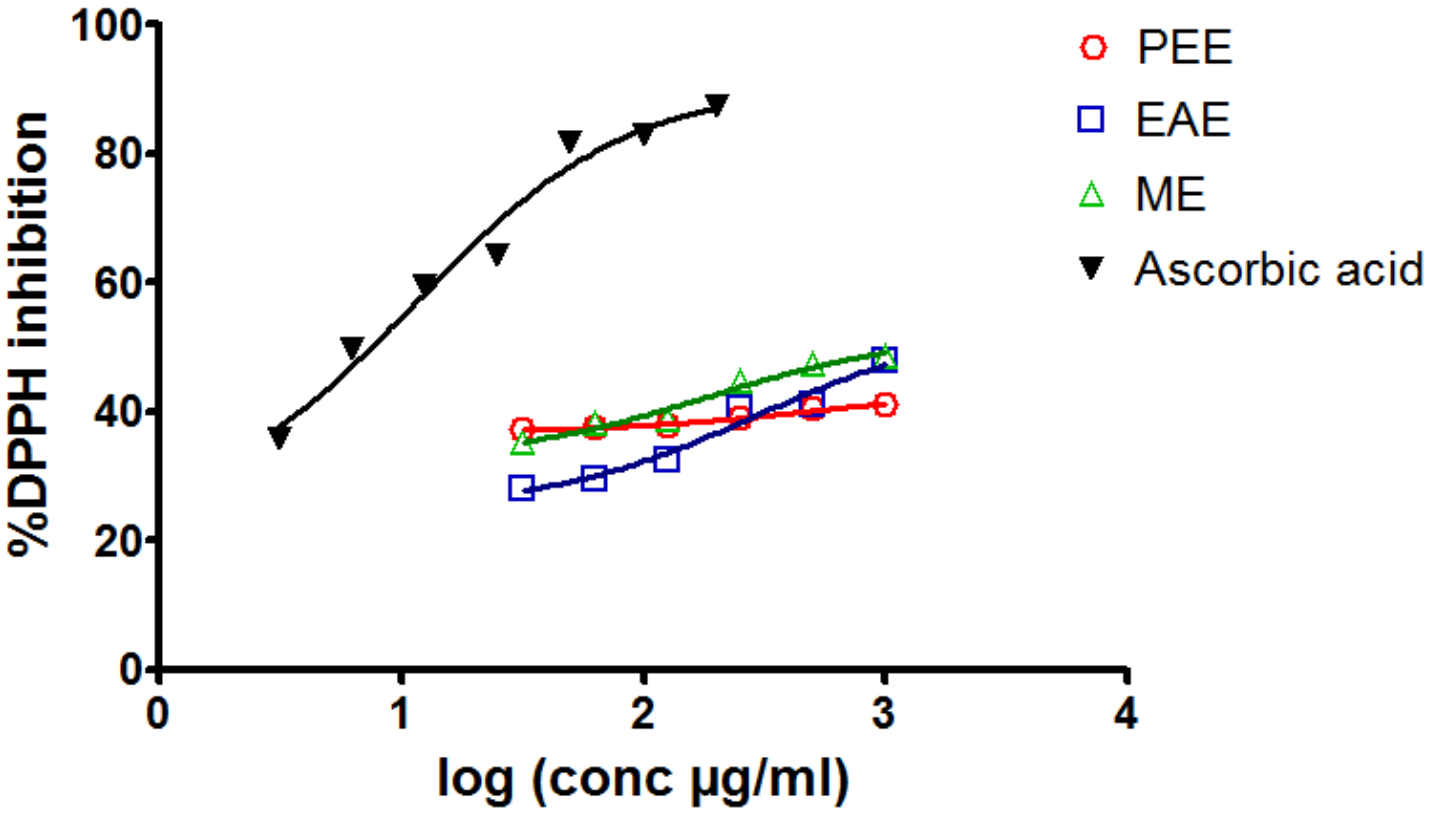

Figure 1

Radical (Free DPPH) scavenging activity of PEE, EAE and ME against ascorbic acid. PEE (Petroleum ether extract); EAE (Ethyl acetate extract); ME (Methanol extract)

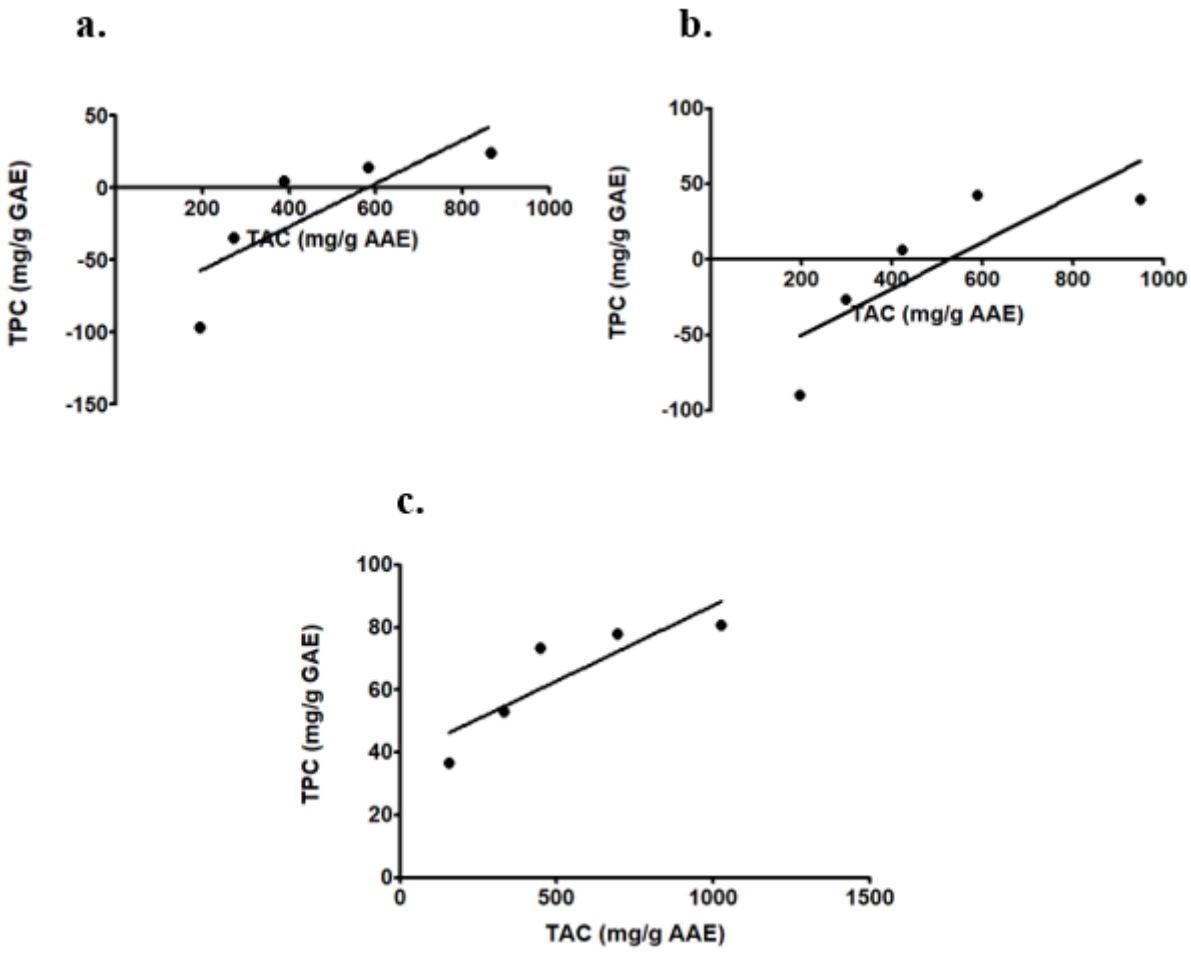

Figure 2

Correlation graph between TPC-Total Phenolic Content and TAC- Total Antioxidant Capacity (TAC) of the extracts: PEE (a), EAE (b) and ME (c).

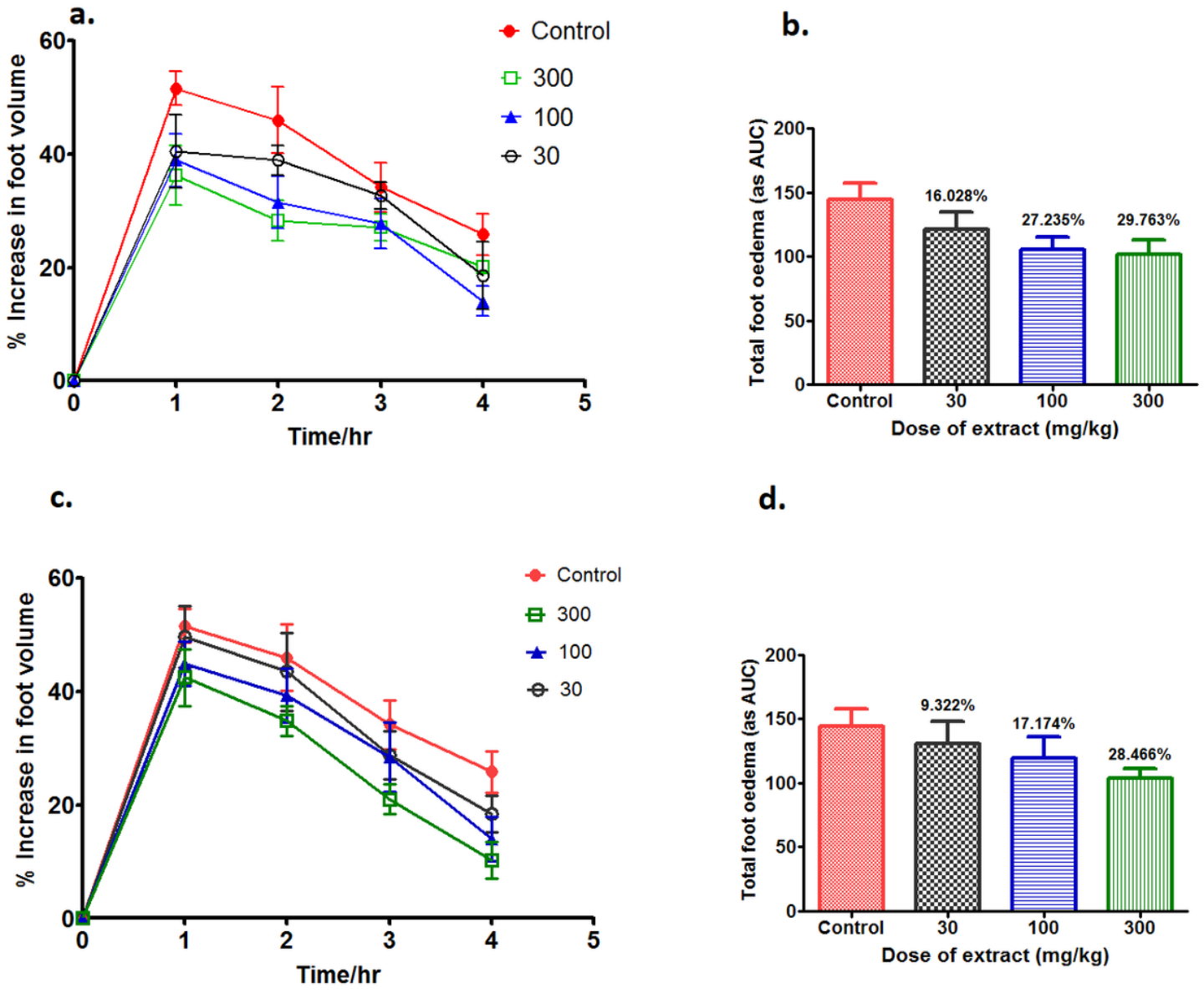

Figure 3

Anti-inflammatory activity of PEE (a,b) and EAE (c,d) (30-300 mg/kg; oral) on timeline curve and the total foot oedema response bars expressed as AUC- area under the curve (b,d) in carrageenan-induced foot oedema in chicks with the percentage inhibitions on the bars, respectively. Values are means ± SEM for five replicate experiments. ***P < 0.0001; **P < 0.001; *P < 0.05 (Data analyzed using One-way ANOVA and afterwards Newman-Keuls post hoc test).

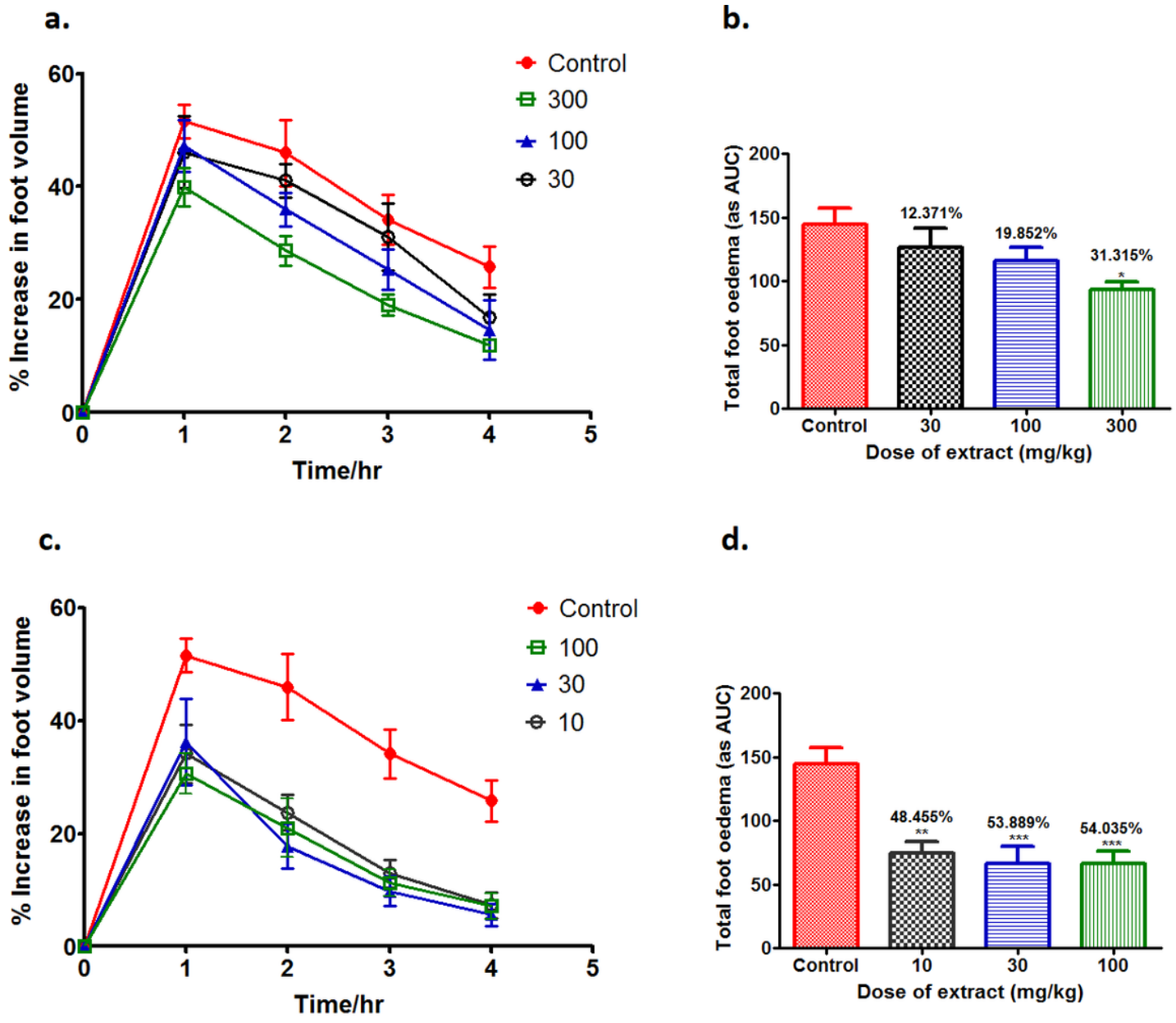

Figure 4

Timeline curve of the anti-inflammatory activity of ME (30-300 mg/kg; oral; a,b) and diclofenac (c,d; 30-100 mg/kg; oral) and the total foot oedema response bars as area under the curve (b&d) with the percentage inhibitions on the bars, respectively. Values are means ± SEM for five replicate experiments. ***P < 0.0001; **P < 0.001; *P < 0.05 (Results analyzed using One-way ANOVA and Newman-Keuls post hoc test).