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Phytochemical Investigation and *in vitro* Antioxidant Activities of *Cleome amblyocarpa* Cultivated in North Sinai.

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Abstract

The development of new pharmaceuticals has mostly benefited from natural products. The widespread medicinal usage of species in Cleomaceae family is extensively researched. *Cleome amblyocarpa* Barr. and Murb. from the family Cleomaceae is a widely grown plant in Sinai Peninsula of Egypt. *C. amblyocarpa* is used in folk medicine as it has analgesic, anti-inflammatory, antibacterial, and antioxidant activities. In this study, the total phenolic content (TPC), total flavonoids content (TFC), and antioxidant activity of crude methanolic extract from whole parts of *Cleome amblyocarpa* growing in Sinai were investigated. Total phenolics and flavonoid contents of the extract were determined by the Folin–Ciocalteu and aluminum chloride methods, respectively. The total phenolic and flavonoid content of *C. amblyocarpa* were 15.63 ± 1.158 µg GA E/mg and 16.1 ± 0.14 µg RE/mg respectively. Two *in vitro* assays (FRAP, and DPPH) were used to determine the antioxidant activity and the ferric reducing ability (FRAP) by *C. amblyocarpa* extract was found to be (65.383 ± 3.88 µM eq/mg) in comparison with Trolox (6.414 ± 1.034 µg/mL). The radical scavenging activity on DPPH (IC_{50} values of 570.6 ± 1.045 µg/mL) was determined using Trolox as a standard drug.

Keywords: *Cleome amblyocarpa*; Antioxidant activity; Phenolic content; flavonoids content; DPPH; FRAP.

1. Introduction

Medicinal plants are an important source of antioxidants **(Williams *et al*., 2004).** As of right now, Cleomaceae family includes 25 genera, with more to be described **(Bayat *et al*., 2018).** Over 200 species of Cleome can be recognized in various regions of the world. It belongs to the largest genus within the family and possesses numerous conventional and medicinal uses **(Singh *et al*., 2018)**. in traditional medicine, cleome species are utilized as analgesic, anti-inflammatory, antibacterial, and antioxidant agents **(Monroy *et al*., 2021).**

C. amblyocarpa Barr. & Murb. is a species of herbaceous plant that belongs to the Cleomaceae family **(Abd-ElGawad *et al*., 2021)**. The plant, which has offensive odor and can be annual or short-lived perennial, is found in North and East Africa, Sinai, Ethiopia, Sudan, Saudi Arabia, Palestine, Iran, and Iraq **(Kamel *et al*., 2010).**

Many bioactive substances have been isolated from *C. amblyocarpa*, such as triterpenoids **(Ahmed *et al*., 2001),** flavonoids, and saponins **(Zaki *et al*., 2020)**. Consequently, many biological activities of this plant have been described, such as anti-leishmanial **(Al Nasr, 2020)**, antifungal **(Hashem, 2011)**, cytotoxic and antioxidant **(Khlifi *et al*., 2020).**

The objective of the present study was to determine the total phenolic and flavonoids contents and antioxidant activity of the total methaolic extract of *Cleome amblyocarpa* whole parts using standard methods.

2. Materials and Methods

2.1. Plant Material:

The whole plant of *C. amblyocarpa* was collected in January 2022 from Northern Sinai Peninsula in Egypt. Prof. Rim Hamdy of the Department of Botany and Microbiology at Cairo University's Faculty of Science confirmed the authenticity of the used plant. The voucher specimen (No. SAA-302) was stored at the Pharmacognosy Herbarium, Suez Canal University, Egypt.

2.2. Materials and Chemicals:

Folin-Ciocalteu reagent (Sigma-Aldrich®, St Louis, MO, USA), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich®, St Louis, MO, USA), FRAP reagent of Trolox was prepared in MeOH from which 5 concentrations were prepared including 12.5, 10, 7.5, 5, 3.75, and 1.875 µg/mL. In a 96-well plate (n=3), 100 µL of freshly created DPPH reagent (0.1% in MeOH) was added together with 100 µL of (Ferric Reducing Antioxidant reagent) (Sigma-Aldrich®, St Louis, MO, USA), Gallic acid (Sigma-Aldrich Co, St Louis, MO, USA), Rutin (Sigma-Aldrich Co, St Louis, MO, USA), Trolox (Sigma-Aldrich Co, St Louis, MO, USA).

2.3. Determination of Total Phenolic Content:

Quantification of total phenolic content (TPC) of *C. amblyocarpa* extract was determined spectrophotometrically via the Folin-Ciocalteu method in accordance with the procedure outlined by the Attard research team **(Attard, 2013)**. A 5 mg/mL solution of the *C. amblyocarpa* in MeOH was prepared. Following the preparation of a 1 mg/ml MeOH stock solution of gallic acid, the following samples were diluted: 46.875, 62.5, 93.75, 125, 187.5, 250, and 375 mg/ml. The process involved combining 100 µL of diluted (1: 10) Folin-Ciocalteu reagent with 10 µL of the *C. amblyocarpa* crude extract in a 96-well microplate. After that, 80 µL of 1M sodium carbonate was added, and the mixture was incubated for 20 min in the dark at room temperature (25 °C). The resulting blue complex colour was measured at 630 nm at the end of the incubation period. The results are presented as µg Gallic acid equivalent/mg extract.

2.4. Determination of Total Flavonoid Content:

The total flavonoids content (TFC) of the *C. amblyocarpa* extract was determined by $AlCl_3$ method as described in **(Kiranmai *et al*., 2011)**, with slight modification in microplates. A solution of final concentration 5 mg/mL was prepared by dissolving the crude extract in MeOH. A stock solution of standard Rutin was prepared at 2000µg/mL in MeOH, from which the following dilutions were prepared: 1000, 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL. A 96-well microplate containing 15 µL of sample/standard, 175 µL of MeOH, and 30 µL of 1.25% $AlCl_3$ were then filled. 30 µL of 0.125 M Sodium acetate was added and incubated for 5 min. The resulting yellow colour was observed at 420 nm after the incubation period. The Results of the samples are presented as µg Rutin equivalent/mg extract

2.5. DPPH Free Radical Scavenging Activity:

The free radical-scavenging activity of *C. amblyocarpa* crude extract was carried out using the method reported in **(Boly *et al*., 2016)**. The crude extract was produced in MeOH at the following

2500, 1250, 625, 3125.5, and 156.25 µg/mL. A stock solution of 100 µg/mL concentration of Trolox was prepared in MeOH from which 5 concentrations were prepared including 12.5, 10, 7.5,5, 3.75, and 1.875 µg/mL. In a 96-well plate (n=3), 100 µL of freshly created DPPH reagent (0.1% in MeOH) was added together with 100 µL of the sample. The reaction was allowed to run at room temperature for 30 min. in dark. The subsequent decrease in DPPH colour intensity was measured at 540 nm at the conclusion of the incubation period. Data are represented as means ± SD according to the following equation:
The percentage inhibition % (PI) of the DPPH radical = ((Average absorbance of blank-average absorbance of the test)/ (Average absorbance of blank) *100)

Microsoft Excel® was used to analyze the data, and Graph Pad Prism 6® was used to obtain the IC_{50} value by converting the concentrations to their logarithmic value and choosing the non-linear inhibitor regression equation (log (inhibitor) vs. normalized response - variable slope equation).

2.6. Ferric Reducing Antioxidant Power (FRAP) Assay:

The FRAP of *C. amblyocarpa* methanolic extract was carried out using the method described by **(Benzie and Strain, 1996)** with minor modifications to be carried out in microplates. 2.5 mg/mL of crude extract was used to prepare the sample in methanol. The following dilutions of Trolox at concentrations of 1000, 900, 800, 700, 600, 500, 400, and 300 µM were produced from a stock solution of 2 mM in MeOH. A freshly prepared TPTZ reagent (300 mM acetate buffer, PH=3.6, 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$, respectively, in a ratio of 10:1:1 v/v/v). In a 96-well plate (n=3), 10 µL of the sample and 190 µL of freshly made TPTZ reagent were combined. The reaction was then allowed to stand at room temperature for 30 minutes in the dark. The ensuing blue colour was detected at 593 nm at the ending of the incubation period. Using the microplate reader FluoStar Omega, the outcomes were recorded.

3. Results and discussion:

3.1 Total Phenolic and Flavonoid Content:

The term "phenolic compound" refers to a broad category of plant compounds which contain an aromatic ring with one or more hydroxyl substituents. They exist in two categories: flavonoids and nonflavonoids (which include lignans, phenolic acids, and coumarins). Considering their biological activity and potential therapeutic benefits, phenolic compounds have recently attracted more attention **(Cosme *et al*., 2020).** The flavonoids are the most abundant group among more than a thousand known structures of natural phenolic compounds, although there are also major numbers of phenylpropanoids, simple monocyclic phenols, and phenolic quinones. In order to determine the possible antioxidant capacity of *C. amblyocarpa* methanolic extracts, it is reasonable to determine the extract polyphenol content as well as its total phenol and flavonoid content. Total phenolic content of *C. amblyocarpa* extract was determined spectrophotometrically using the Folin–Ciocalteu reagent **(Attard, 2013)**. The estimated linear equation was $y = 0.004x - 0.027$ ($R^2 = 0.995$) **(Figure 1)** based on the calibration curve of gallic acid. The methanolic extract of *C. amblyocarpa* contained 15.63 ± 1.158 µg GAE/mg of total phenols. Rutin was used as a reference standard and the $AlCl_3$ reagent **(Kiranmai *et al*., 2011)** was used to measure the total flavonoid content of the *C. amblyocarpa* extract. The linear equation, derived from the rutin calibration curve, was $y = 0.002x - 0.013$ ($R^2 = 0.999$) **(Figure 2)**. According to the equation above, the methanolic extract of *C. amblyocarpa* contained 16.1 ± 0.14 µg R E/mg of plant extract of total flavonoids contents.

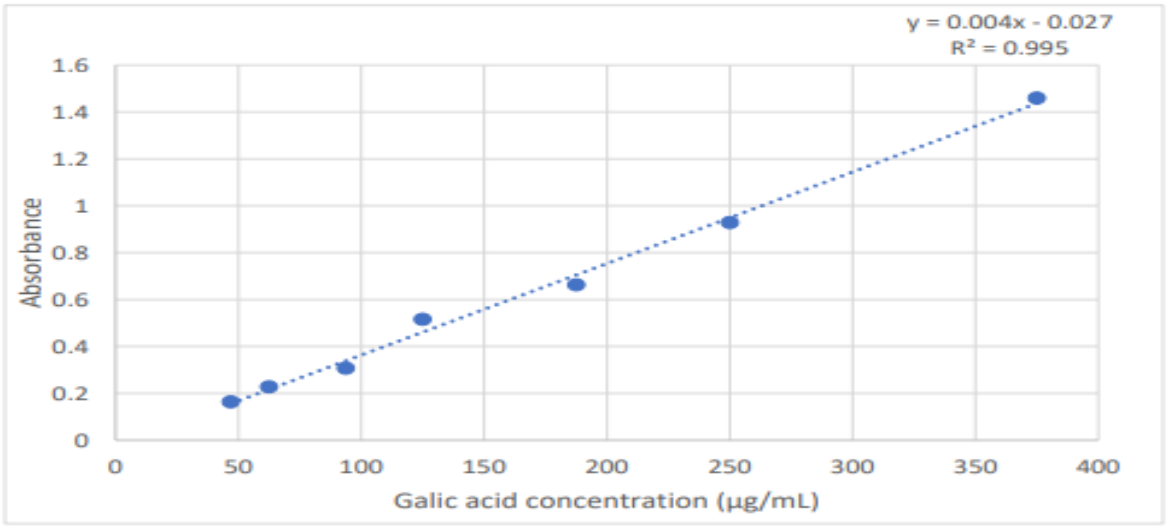

Figure 1: Calibration curve of standard gallic acid for determination of total phenolics content of the methanolic extract of *C. amblyocarpa*

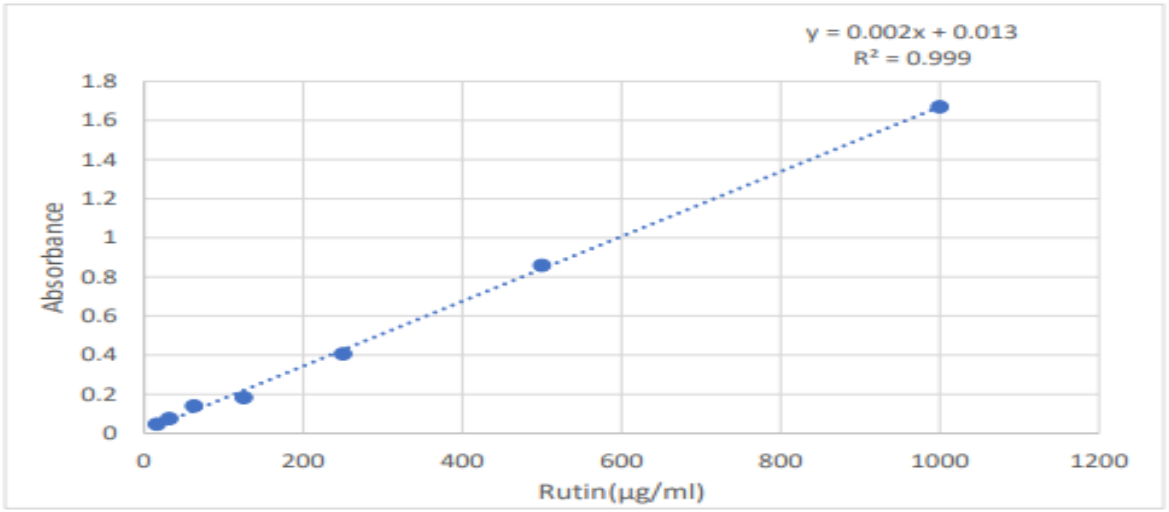

Figure 2: Calibration curve of standard rutin for determination of total flavonoids content of the methanolic extract of *C. amblyocarpa*

3.2 Evaluation of *in-vitro* antioxidant activity of *C. amblyocarpa* extract:

Antioxidants are substances that defend live cells from damage caused by unstable chemicals known as free radicals **(El-Beltagi *et al*., 2018)**. Antioxidant molecules can delay the synthesis of other molecules or prevent them from oxidation. When electrons are transferred from one substance to another, the chemical reaction is referred to as oxidation. Oxidation processes generate free radicals, which set off a cascade of harmful cell-damaging events. By limiting other oxidation events and destroying free radical intermediates, antioxidants prevent these chain reactions **(Santos *et al*., 2019).** In this study, two suggestive assays (DPPH, FRAP) were used to evaluate the antioxidant potential of *C. amblyocarpa* crude extract. The result showed that the *C. amblyocarpa* crude extract had definite scavenging activities with DPPH and FRAP assays displaying a dose-dependent scavenging rate.The Trolox solution of concentration (300-1000 µg/mL) at 593 nm with a regression co-efficient (R_2) = 0.998. The plot has a slope (m) = 0.001 and intercept = 0.111. The equation of standard curve is y = 0.001x + 0.111 (Figure 3). IC_{50} value of *C. amblyocarpa* crude extract = 570.6± 1.045 µg/ml. it also exhibited marked activity in DPPH radical scavenging assay compared to Trolox reference standard with IC_{50} = 6.414 ± 1.034 µg/ml. The graphical representation of the DPPH is presented in **(figure 4)**. The ferric reducing ability of the *C. amblyocarpa* is presented as 65.383± 3.88 µMeq/mg in comparison to Trolox reference standard.

4. Conclusion:

In this study, The plant extract's TPC for *C. amblyocarpa* was 15.63 ± 1.158 µg GA E/mg. The $AlCl_3$ technique was used to calculate the total flavonoid content (TPC) of the methanolic extract of *C. amblyocarpa*. at 16.1 ± 0.14 µg RE/mg of plant extract, *C. amblyocarpa* had a TFC. The antioxidant activity was assessed using two suggestive tests (FRAP and DPPH). In contrast to Trolox (6.414 ± 1.034 µg/mL), *C. amblyocarpa* extract shown a significant antioxidant capacity-FRAP (65.383 ± 3.88 µM eq/mg). Comparing the radical scavenging activity of DPPH to Trolox as a reference standard, the IC_{50} values were 570 ± 1.045 µg/mL.

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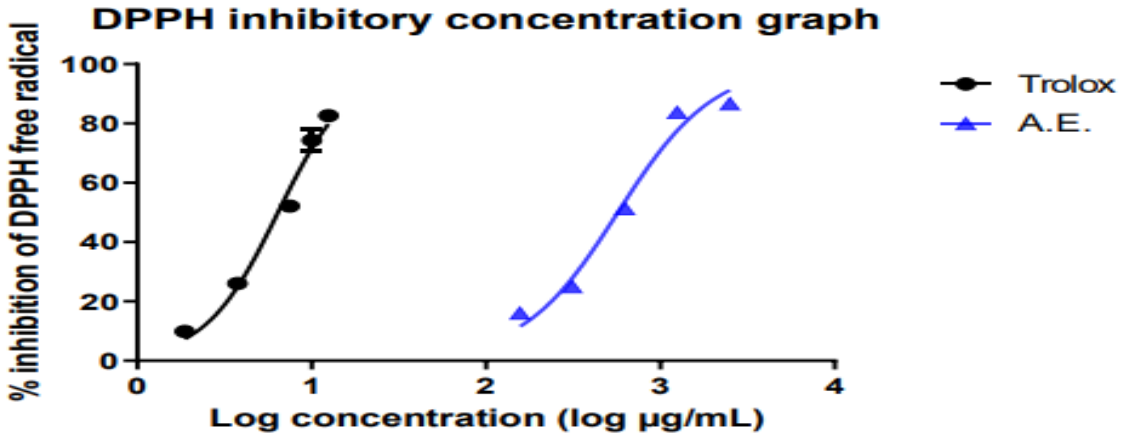

Figure 3: *In vitro* antioxidant assay for standard Trolox

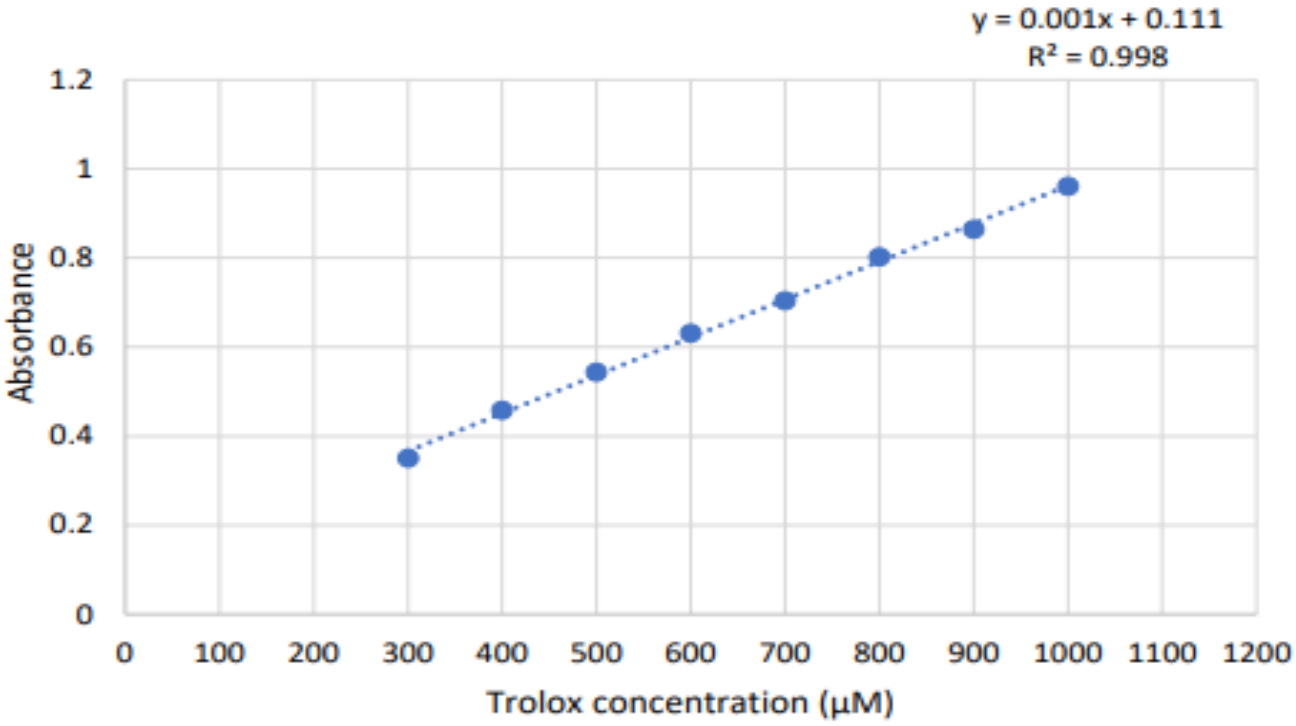

Figure 4: DPPH Free radical scavenging activity of the MeOH whole plant extract (A.E) of *C. ambyocarpa*. Trolox was included as a positive control. Each value is the mean ± standard deviation.

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6. Conflict of interest:

The authors report no declaration of conflict of interest.

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