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PHARMACOGNOSIE

High in vitro Antioxidant Capacities of Algerian *Cleome arabica* Leaves' Extracts

Hautes capacités antioxydantes in vitro des extraits de feuilles de *Cleome arabica* d'Algérie

F. Seglab · C. Hamia · I. Khacheba · A. Djeridane · M. Yousfi

Abstract The aim of this study was to evaluate the antioxidant capacities of *Cleome arabica* leaves' extract collected on two seasons in the town of Laghouat steppe region of Algeria. Five common tests for measuring antioxidant capacities were used to compare with three standard antioxidants: measurement of free radical scavenging activity with 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) and 2,2′-azinobis(3-ethylbenzo-thiazo-line-6-sulfonate) ($ABTS^{\bullet+}$) radical cation, measurement of total antioxidant capacities with phosphomolybdenum, ferric reducing, and cupric reducing methods. The amount of the phenolic compounds was carried out by the quantification of total phenolic, total flavonoid, and condensed tannin contents in three organic solvents with varying polarities. The results show that the ethyl acetate is the best extractor solvent of flavonoids, while petroleum ether has the ability to extract more of terpenes. The values of quantification ranged from 0.341 to 0.751 mg of gallic acid equivalent/g of dry matter, from 0.172 to 0.682 mg of quercetin equivalent/g of dry matter and from 0.172 to 0.332 mg of catechin equivalent/g of dry matter for the total phenolic, flavonoids, and the condensed tannins, respectively. All the extract shows strong antioxidant activity, whose best are found in the ABTS and DPPH assay with IC_{50} values of 0.01 and 0.017 mg/ml, respectively, in a different season. These results suggest that the level of antioxidant activity in this plant varies to a great extent. They also suggest that phenolics in this plant provide substantial antioxidant activity. Upon achievement of this survey, an extra benefit of this medicinal plant may be found.

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Keywords *Cleome arabica* · Ethyl acetate extracts · Phenolic compounds · Antioxidant capacity · PCA

Résumé Le but de cette étude était d'évaluer les capacités antioxydantes des extraits de feuilles de *Cleome arabica* récoltées à deux saisons de la région de la steppique algérienne de Laghouat. Cinq tests des capacités antioxydantes ont été utilisés, comparés à trois antioxydants standards : mesure de l'activité de piégeage des radicaux libres avec le radical 1,1-diphényle 2-picrylhydrazyle (DPPH•) et le radical cation 2,2′-azinobis (3-éthylbenzo-thiazo-6-sulfonate) ($ABTS^{\bullet+}$), la mesure des capacités antioxydantes totales avec les méthodes du phosphomolybdate, la réduction du fer ferrique et la réduction du cuivre cuivrique. La quantité de composés phénoliques a été déterminée par la quantification de la teneur totale en composés phénoliques, de la teneur totale en flavonoïdes et de la teneur en tanins condensés dans trois solvants organiques de polarités variables. Les résultats montrent que l'acétate d'éthyle est le meilleur solvant d'extraction des flavonoïdes, tandis que l'éther de pétrole a la capacité d'extraire davantage de terpènes. Les valeurs de quantification variaient de 0,341 à 0,751 mg équivalent en acide gallique/g de la matière sèche, de 0,172 à 0,682 mg équivalent en quercétine/g de la matière sèche et de 0,172 à 0,332 mg équivalent en catéchine/g de la matière sèche pour les phénols totaux, les flavonoïdes et les tanins condensés respectivement. Tous les extraits montrent une forte activité antioxydante, les meilleurs étant trouvés dans les tests ABTS et DPPH avec des valeurs d'IC_{50} de 0,01 et 0,017 mg/ml respectivement au cours d'une saison différente. Ces résultats suggèrent que le niveau d'activité antioxydante de cette plante varie dans une large mesure. Ils suggèrent également que les composés phénoliques de cette plante fournissent une activité antioxydante substantielle. Après avoir réalisé cette étude, un avantage supplémentaire de cette plante médicinale pourrait être trouvé.

Mots clés *Cleome arabica* · Extraits d'acétate d'éthyle · Composés phénoliques · Capacité antioxydante · ACP

Introduction

In recent years, the world of biological and medical science has been convinced by a new concept of oxidative stress, that is, a situation where the cell no longer controls the excessive presence of toxic oxygen radicals, a situation which researchers implicate in most human diseases [1]. Plant materials have various beneficial medicinal effects which are typically due to the combinations of phytochemical compounds present in the plant, and their action is exceptional to specific plant species or groups [2,3]. Phenolic compounds are a class of naturally occurring pigments with ubiquitous distribution in plant kingdom [4]. They display a remarkable biological property, which may have an important role in the reduction of oxidative stress. Many antioxidants are proven to undergo an electron transfer mechanism upon exerting their antioxidant functions [5]. Some workers have attempted to establish quantitative correlations between the antioxidant activities and redox potentials for reductant antioxidants. *Cleome arabica* belongs to Capparaceae family that includes 150 species widely grown in tropical and subtropical regions of the world, which is also widespread in North Africa. This plant metabolizes a large number of secondary metabolites, mainly flavonoids, alkaloids, glycosides, organic acids, and glucosinolates [6,7]. It has been used in traditional medicine in the treatment of scabies and inflammation [6,8-10] as well as its leaves, which contain a number of glucosylated and rhamnosylated flavonols and possess anti-inflammatory activity which are used for the treatment of abdominal and rheumatic pains [11]. It also possesses antimicrobial, antifungal, and cytotoxic activity against P388 cells [12,13].

Despite the abundant research that has been carried out on the potential health benefits of *Cleome arabica*, little research was done about their biological activities, especially the antioxidant activity [4,14].

Taking into account the complexity of the oxidation processes, there is no single method of reflecting the antioxidant profile of a sample; therefore, the response obtained by means of different and complementary tests must be combined. The aim of this study is to evaluate phenolic compounds, in vitro antioxidant effects of *Cleome arabica* extracts collected in different seasons in the town of Laghouat in the steppe region of Algeria. In this work, we aim to assess the antioxidant potential of *Cleome arabica samples* using five chemical tests, (ABTS; ferric reducing antioxidant power, FRAP, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), phosphomolybdenum complex, and cupric reducing antioxidant [CUPRAC]). To find the correlation between them in different seasons and compare them with those of butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and ascorbic acid (VC) have been extensively used as standard antioxidants in the experiments, which can offer a wider picture of the antioxidants present in biological samples as it rather than considers the effect of single compounds, it considers the additive and synergistic effects of all antioxidants, and may, therefore, be useful to study the potential health benefits of antioxidants on oxidative stress-mediated diseases [15,16]. To the best of our knowledge, the antioxidant activities with various methods of Algerian *Cleome arabica* was never been investigated. Also, this work is a continuity of studies on *Cleome arabica* [17] and other antioxidant studies on Algerian plants realized in our laboratory [18–22].

Materials and Methods

Plant Material

The plant (*Cleome arabica*) was collected in two different seasons: January and November 2015. The two samples were harvested at Laghouat city in the steppe region of Algeria. A voucher specimen was deposited into the laboratory of Fundamental Sciences, University of Laghouat. The leaves were dried, grinded, and kept until analysis.

Reagents

All chemicals reagents were from Sigma (USA), Aldrich (Milwaukee, USA), Fluka Chemie (Buchs, Switzerland), and Merck (Germany). The used solvents were of analytical grade.

Preparation of Extracts

The fresh *Cleome arabica* material leaves were oven-dried and grinded. The lipid was removed by Soxhlet extraction with petroleum ether for 24 h. After that, the remaining powder was macerated in hydroalcoholic solvent system with 100 ml of 80/20 (v/v) (methanol/water) for 24 h at room temperature. After evaporation of water under reduced pressure, the organic fraction was extracted with a series of solvents of increasing polarity as hexane and dichloromethane to remove the pigments and lipid compounds. Then, the extraction was done with ethyl acetate until exhaustion. Organic extract was evaporated to dryness under reduced pressure at 45 °C. Dry fractions were stored in ethanol at 4 °C until use.

Determination of Total Phenolic Content

The total phenolic content was spectrophotometrically determined using the Folin–Ciocalteu method. This test is based on the oxidation of phenolic groups by phosphotungstic and

phosphomolybdic acids (FC reagent). This reagent, based on the early work of Singleton & Ross method [23], is a colorimetric oxidation/reduction method for phenolic compounds. The intensity of light absorption at that wavelength is proportional to the concentration of phenols. Briefly, a 100 µl of the diluted sample was added to 500 µl of Folin–Ciocalteu reagent. After 3 min, 2 ml of saturated sodium carbonate solution (2%) was added. The products of the metal oxide reduction have a blue color that exhibits a broad light absorption with a maximum at 764 nm. The calibration curve was prepared with Gallic acid solutions, and the results are given as Gallic acid equivalents (GAE).

Determination of Total Flavonoid Content

The total flavonoid content was determined using a spectrophotometric method of Lamaison and Carnat [24] with minor modification by Floegel et al. [25]. Briefly, 1 ml of sample or standard (quercetin) was mixed with 1 ml (w/v) of aluminum chloride ($AlCl_3$) 2%. Absorbance of the colored flavonoid–aluminum complex was measured immediately at 430 nm versus a blank. The total flavonoid content of the *Cleome arabica* L. extract was expressed as mg quercetin equivalent (QE)/100 g of dry weight material. All samples were analyzed in triplicate.

Determination of Tannin Condensed Content

The condensed tannins are determined by the vanillin method with slight modification [26,27]. This method is based on the ability of vanillin to react with condensed tannin units in the presence of acid to produce a colored complex measured at 500 nm. The reactivity of vanillin with tannins implies only the first unit of the polymer. 5 ml of reagent comprises 0.5% vanillin and 4% HCL prepared in the methanol added to 1 ml of diluted extract. The reagent and diluted extract preincubated in a water bath at 20 °C for 10 min before the reaction starts, and then the mixture is incubated for 15 min. The reading of the absorbance is carried out in a Visible/UV spectrophotometer of Shimadzu type 1601, at a wavelength of 500 nm against a white. A calibration curve is performed in parallel under the same operating conditions using catechin as a positive control.

Evaluation of Antioxidant Capacities

Phosphomolybdenum Assay

The antioxidant activity of the *Cleome arabica* L. extract was evaluated by the phosphomolybdenum method. The assay is based on the reduction of molybdates Mo (VI) to molybdenum Mo (V) Mo by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. The followed scheme is to combine 0.1 ml extract with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). In case of blank, 0.1 ml of distilled water was used in place of the test sample. The tubes containing the reaction solution were capped and incubated in a boiling water bath at 70 ° C for 90 min. We subject the samples to a fast cooling before measuring the absorbance of the solution at 695 nm using a spectrophotometer [28]. The antioxidant capacity of each sample was expressed as ascorbic acid (A.A) equivalent. The values are presented as the means of triplicate analysis.

Ferric Ion Reducing Antioxidant Power (FRAP) Assay

The ferric ion reducing antioxidant power (FRAP) method was used to measure the decreasing capacity of *Cleome arabica* L. extracts; this method was carried out with slight modifications [29,30]. FRAP reagent was prepared by mixing acid buffer, TPTZ solution and ferric chloride solution at the volume ratio of (10:1:1) respectively.

The following protocol is used for the buffer acid (pH = 3.5): first dissolve 0.310 g of $CH_3COONa, 3H_2O$ in a 100-ml flask, then add 1.6 ml of pure acetic acid, and complete with distilled water up to gauge. The TPTZ solution is prepared by dissolving 157 mg of this compound in 50 ml distilled water and 0.165 ml of HCl 40 mmol/l. For the solution of ferric chloride (20 mmol/l), we have dissolved 0.2723 g of $FeCl_3, 6H_2O$ in a 50-ml flask and added with distilled water.

For samples to be tested, they were mixed in the same proportions as to plot the standard curve, the reagent (1 000 µl), and the solution of the test compound (100 µl). The optical density is read after 1 h at 593 nm. The ability "electron donor" of the sample is measured by the change in color due to the formation of the complex (Fe^{2+} TPTZ) and the equivalent in Fe^{2+} is determined.

All measurements were repeated three times. Total antioxidant capacity in the measuring systems, expressed as VC equivalents, was calculated. Correlation coefficient (R^2) for the test curve ranged between 0.99 and 1.00.

Copper Reduction Capacity Assay (CUPRAC)

The evaluation of the reducing capacity of our samples was carried out using the test CUPRAC. It is based on the following by the reduction of increased absorbance of neocuproine complex (Nc)-ions cupric [Nc2-Cu2^+]. Indeed, in the presence of an antioxidant, the copper complex neocuproine is reduced and this reaction was quantified spectrophotometrically at a wavelength of 456 nm [31]. The preparation scheme for the measurement of the antioxidant properties

was the following: 1 ml of ammonium acetate buffer (1 M, pH = 7 > *m* = 19.66 g in 250 ml water) was mixed with 100 µl of diluted extract to be tested, 500 µl of neocuproine (7, 4 mM ≥ *m* = 0.039 g in 25 ml ethanol), and 500 µl of $CuCl_2$-$2H_2O$ 10.1 mM (0.01 m ≥ *m* = 0.1722 g in 100 ml water). The mixture is incubated for 1 h, and then the reduced complex [−Nc Cu2 +] was quantitated spectrophotometrically at a wavelength of 456 nm against a blank.

DPPH Free Radical Scavenging Activity Assay

Antioxidant activity of *Cleome arabica* L. extract with respect to the radical DPPH was evaluated spectrophotometrically by following the reduction of this radical which is accompanied by its passage from the violet color to the yellow color measurable at 517 nm. According to the method reported by Cotelle et al. [32] with minor modifications [33,34]. Briefly, ethanolic solution extract and standard solutions (control) were prepared and added to a 250 µM of DPPH ethanolic solution; the mixtures were shaken vigorously and left to stand in the dark for 30 min at room temperature, and then absorbance was read at 517 nm. Radical scavenging capacity was expressed as percentage effect (E%) and calculated using the following equation:

$$Percentage\ effect\ (E\%) = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right) \times 100$$

Different sample concentrations were used in order to obtain antiradical curves for calculating the EC_{50} values. Antiradical curves were plotted referring to concentration on the *x*-axis and their relative scavenging capacity on the *y*-axis. The EC_{50} values were processed using six or plus of points.

ABTS Radical Cation Decolorization Assay

ABTS assay was described by Re et al. and Serpen et al. [35,36] with minor modification. The radical cation $ABTS^{+}$.occurs immediately after the addition of 100 µl. The $ABTS^{\bullet+}$ radical cation was generated by chemical oxidation with potassium persulfate of a potassium persulphate $K_2S_2O_8$ solution (70 mM). The mixture is brought to room temperature and the dark for 24 h before use. 1 ml of this solution was diluted of a buffered solution at pH 6.8 to 7.0 phosphate prepared by dissolving 0.4477 g of sodium hydrogen phosphate hydrate 12 times (Na_2HPO_4-$12H_2O$), 0.195 g of hydrated sodium dihydrogen phosphate (NaH_2PO_4-$2H_2O$), and 2.25 g of chloride sodium (NaCl) in 250 ml of distilled water. The optical density of the pre-solution incubated at 37 °C for 15 min is adjusted to (0.70 ± 0.002) at 734 nm.

100 µl of each diluted extract in distilled water is admixed with 1 ml of the ABTS + solution. The percentage inhibition (PI) of ABTS + for each sample was calculated according to the formula below:

$$Percentage\ inhibition\ (PI\%) = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right) \times 100$$

Also, we tested vitamin C, TBHQ, and BHT as standard. The %AI of the extracted phenols was expressed as IC_{50}, with the IC_{50} being defined as the concentration of test sample required to inhibit the formation of active radicals by 50%.

Results and Discussion

Content of Total Phenolics, Flavonoids, and Condensed Tannins

The extraction solvents carry off non-phenolic substances such as sugars, dyes, and proteins which may interfere during any phenolic evaluation [37]. Aqueous ethanolic extraction proved to be the most effective method for extracting phenolic materials and other antioxidative compounds [38].

The results of the total phenolic content in the ethyl acetate fraction ranged between 0.341 and 0.751 mg EAG/g for the dry matter, 0.170 and 0.490 mg EAG/g for the dichloromethane fraction, and between 0.060 and 0.453 mg EAG/g for the petroleum ether fraction. The total phenolic content in many samples tested in this study was higher than reported for the other medicinal [39]. But we marked that the total phenolic content in the tested plant was lower than those reported for most of the other Asian medicinal and common dietary plants [14,15].

The amount of flavonoid content in the ethyl acetate fraction ranged from 0.172 to 0.682 mg EQ/g. The results of total phenolic, flavonoid, and tannin condensed contents are shown in table 1. The greatest amount of flavonoids was recorded in the ethyl acetate fraction but no quantity was registered in the dichloromethane and petroleum ether fraction.

Calycopterine; Quercetin glucoside; Quercetin 3,7-diglucoside; Kaempferol 3-G-7-Rhamnoside; Quercetin 7-Rhamnoside; Apigenin 6,8-di-CGlycoside; Kaempferol 3,7-diRhamnoside; Quercetin 3-G-7-rhamnoside; Kaempferol 3′methoxy-3,7 diRhamnoside; Kaempferol 7-Rhamnoside; Isorhamnetin.

For the tannins content, we observe the same result as flavonoids exist only in the ethyl acetate fraction. The tannins assay method used is that of vanillin described by Hagerman [27]. The results of the condensed tannins in the ethyl acetate fraction varied between 0.172 and 0.332 mgEC/g. We conclude that the ethyl acetate is the best extractor of flavonoids and the condensed tannins.

Measurement of Antioxidant Capacity Expressed as Vitamin C Equivalents

The antioxidant capacities of *Cleome arabica* L. extract are summarized in table 2. The highest antioxidant capacities were detected by ABTS with extract series concentration from 0.1 to 0.8 mgGAE/g, and EC_{50} values ranged from 0.371 to 0.909 mg VCE/100 g is better than that of BHT (0.021 mg/ml), TBHQ (0.023 mg/ml), and ascorbic acid (0.057 mg/ml). However, the lowest antioxidant activity was registered between (9.265 and 12.215 VCE mg/100g), (2.923 and 11.305 mg VCE/100 g), and (8.459 and 33.759 mg VCE/100 g) for CUPRAC assay, FRAP, and phosphomolybdenum complex (PM-complex) assay, respectively. There are different reasons that lead to these differences. One of these reasons could be that CUPRAC method can determine the antioxidant substances containing sulfhydryl group (SH), for example, the proteins. Not all methods measure protein thiols or smaller molecule -SH compounds of different origins such as GSH, with FRAP. Also, the

Table 1 Total amount of phenolic compound, flavonoids, and tannins condensed of *Cleome arabica* leaves' extract

Samples N°	Total phenolic content[a] mg/g	Total flavonoid content[b] mg/g	Total tannin content[c] mg/g
N1	0.686 ± 0.01	0.329 ± 0.002	0.332 ± 0.007
N2	0.751 ± 0.02	0.682 ± 0.003	0.274 ± 0.013
N3	0.341 ± 0.01	0.204 ± 0.005	0.188 ± 0.014
N4	0.362 ± 0.00	0.329 ± 0.003	0.302 ± 0.002
N5	0.409 ± 0.01	0.271 ± 0.004	0.172 ± 0.008
J1	0.624 ± 0.02	0.314 ± 0.002	0.328 ± 0.014
J2	0.470 ± 0.01	0.245 ± 0.001	0.190 ± 0.007
J3	0.347 ± 0.01	0.172 ± 0.002	0.187 ± 0.01
J4	0.595 ± 0.03	0.403 ± 0.001	0.329 ± 0.006
J5	0.449 ± 0.01	0.261 ± 0.002	0.213 ± 0.017
J6	0.415 ± 0.01	0.223 ± 0.003	0.216 ± 0.017

[a] mg of gallic acid/g of sample (mgGAE/g(
[b] mg of quercetin/g of sample (mgQE/g(
[c] mg of catechin/g of sample (CE/g(

Table 2 Antioxidant activity of examined *Cleome arabica* L. ethyl acetate extracts and standards determined by DPPH, ABTS, FRAP, CUPRAC, and PM assays

Samples' code	CUPRAC (g/l)	PM (g/l)	FRAP (g/l)	DPPH (g/l)	ABTS (g/l)
N1	0.323 ± 0.004	0.446 ± 0.027	0.158 ± 0.002	0.015 ± 0.000	0.013 ± 0.000
N2	0.22 ± 0.009	0.262 ± 0.006	0.232 ± 0.0025	0.01 ± 0.000	0.009 ± 0.000
N3	0.24 ± 0.0004	0.248 ± 0.006	0.099 ± 0.001	0.013 ± 0.000	0.01 ± 0.000
N4	0.166 ± 0.002	0.162 ± 0.020	0.053 ± 0.001	0.010 ± 0.000	0.009 ± 0.000
N5	0.261 ± 0.009	0.243 ± 0.003	0.094 ± 0.001	0.017 ± 0.000	0.01 ± 0.000
J1	0.296 ± 0.010	0.29 ± 0.002	0.117 ± 0.002	0.017 ± 0.000	0.01 ± 0.000
J2	0.265 ± 0.006	0.273 ± 0.011	0.104 ± 0.000	0.023 ± 0.000	0.01 ± 0.000
J3	0.255 ± 0.008	0.314 ± 0.001	0.12 ± 0.005	0.018 ± 0.000	0.01 ± 0.000
J4	0.24 ± 0.002	0.238 ± 0.017	0.097 ± 0.003	0.02 ± 0.001	0.009 ± 0.000
J5	0.27 ± 0.010	0.287 ± 0.003	0.119 ± 0.001	0.018 ± 0.001	0.01 ± 0.001
J6	0.25 ± 0.003	0.303 ± 0.010	0.11 ± 0.003	0.025 ± 0.002	0.009 ± 0.001
BHT	0.093 ± 0.002	2.495 ± 0.053	0.145 ± 0.001	0.168 ± 0.004	0.021 ± 0.000
TBHQ	0.2845 ± 0.004	0.593 ± 0.006	0.056 ± 0.000	0.04 ± 0.001	0.023 ± 0.000
VC	0.366 ± 0.002	0.443 ± 0.009	0.109 ± 0.000	0.001 ± 0.000	0.057 ± 0.005

N: November, J: January

CUPRAC method is advantageous over FRAP since the redox chemistry of copper(II) — as opposed to that of chemically inert high-spin ferric ion having half-filled d-orbitals in its electronic configuration — should involve faster kinetics. The bis-(neocuproine) copper (I) cation chromophore is soluble both in water and organic media; therefore, the CUPRAC method is capable to assay both hydrophilic and lipophilic antioxidants. In this experiment, the ABTS method was used to confirm the results from the DPPH method assay since both methods are based on a similar antioxidant mechanism and the extracts used in both tests were ethanol soluble. All sample extracts are comparable without significant differences.

For the case of DPPH and ABTS radicals, the mechanism (particularly in ethyl acetate) is very often electron transfer than the other methods; furthermore, the antioxidant activity of *Cleome arabica* does not change with the different seasons of the year.

Antioxidant Capacity of the Relevant Standards

Some relevant organic compounds are used as antioxidant standards, including ascorbic acid, and BHT and TBHQ were comparatively evaluated for their ranking of antioxidant power by DPPH, ABTS, FRAP, PM-complex, and CUPRAC assays. A lower IC_{50} and EC_{50} values correspond to a larger scavenging activity. As shown in table 2, the DPPH radical scavenging activities of these reference compounds were comparatively evaluated and ascorbic acid possessed the highest radical scavenging activity, 0.001 g/l as compared with TBHQ 0.04 g/l and BHT 0.168 g/l; and the ranks of the scavenging activity were found in similar trends with DPPH and PM-complex assays, ascorbic acid > TBHQ > BHT. On the contrary, the ranks of the scavenging activity were found in similar trends with CUPRAC and ABTS, BHT > TBHQ > ascorbic acid. The linearity of calibration curves allowed quantification of antioxidant activity using any of the standards listed above. DPPH, ABTS, PM-complex, CUPRAC, and FRAP values (Table 2) for the antioxidant activity of these standards were used for investigating the correlation coefficients. Antioxidant activity of many samples of *Cleome arabica* L. extract tested in this study was higher than reported for most of the other medicinal plants [40–44]. These results of the study are in good accordance with previous studies (Table 3).

We have observed rapid and strong inhibition of both DPPH radical and ABTS radical cations, after the addition of *Cleome arabica* L. extract. Compared to other methods, from the methodological point of view, the DPPH method is suggested as easy and perfect with regard to measuring the antioxidant activity of fruit and vegetable juices or extracts. The results are highly reproducible and comparable to other free radical scavenging methods such as ABTS [45].

Correlation Between Results of Antioxidant Activities and Phytochemical Constituents

It is clear from this study that the different methods used for the determination of antioxidant capacity are highly correlated. Correlation analysis is used for phytochemical contents with IC_{50} values of radical scavenging and/or antioxidant ability of extract. IC_{50} of ABTS, CUPRAC, and FRAP showed a significant correlation (r: 0.713, 0.948, 0.985) with PM-complex. While nonsignificant correlation was observed with DPPH, a positive correlation was also observed between flavonoids and total polyphenolic (r: 0.781), confirming that flavonoids are the most important group of phenols in plants [46]. The total phenolic and flavonoids showed significant correlation (r: 0.646, 0.782) with FRAP, respectively, but no significant correlation observed between flavonoids

Table 3 EC_{50} of *Cleome arabica* L extracts compared with previous studies

Plant	DPPH	ABTS	References
Satureja thymbra	0.391 ± 0.45 mg/ml	0.178 ± 0.80 mg/ml	[40,37]
Lemon essential oils	6.68 ± 0.33 mg/ml	2.97 ± 0.18 mg/ml	[27]
Avocado	41.2 ± 6.4 mg VCE/100 g	70.0 ± 10.1 mg VCE/100 g	[22]
Apple	143.7 ± 47.3 mg VCE/100 g	158.7 ± 35.6 mg VCE/100 g	[22]
Lemon	101.2 ± 2.0 mg VCE/100 g	145.8 ± 13.7 mg VCE/100 g	[22]
Cleome arabica	13.15 ± 0.01 mg/l	/	[11]
Anvillia radiata	≤ 0.380 mg/ml	0.486 ± 0.038 mg/ml	[38,41]
Cleome arabica	0.00488 mg/ml	/	[22]
Rumex vesicarius	0.140 ± 0.007 mg/ml	/	[38]
Nepeta nepetella	1.45 ± 0.07 mg/ml	/	[39,42]
Lemon balm	16.35 ± 0.82 mg/ml	10.37 ± 0.62 mg/ml	[27]
Dark tea	0.013 ± 0.001 mg/ml	/	[43]
Brick tea	0.043 ± 0.002 mg/ml	0.027 ± 0.001 mg/ml	[44]

(CUPRAC, DPPH, PM-complex, ABTS). This observation may suggest that the reducing power exhibited by the different fractions is not only a function of flavonoids but also of the presence of other constituents with an antioxidant potential [47]. In addition, correlations among antioxidant activity based on DPPH, ABTS, CUPRAC, PM-complex, and FRAP assays were positively high and ranged between 0.413 and 0.985. The results show that when all standard materials were relatively analyzed by statistics, there was a positive and greatly significant relationship for PM-complex and CUPRAC (r = 0.985). Statistically significant correlations were also noted between PM-complex and ABTS values (r = 0.966) and ABTS and CUPRAC values (r = 0.948). Furthermore, we registered a positive correlation between PM-complex and FRAP (r = 0.713) and REC and FRAP values (r = 0.692). This strong correlation is due to the fact that these four methods are based on the generation of a free radical by different mechanisms followed by the detection and quantification of an end point in the reaction. The addition of an antioxidant inhibits the development of the end point, an inhibition that is an expression of the antioxidant capacity of the sample studied [48]. Moderately positive correlation has been found between total flavonoids content vs FRAP: r = 0.646. These results indicate that the antioxidant activities proved by these tests are ensured, probably by the same active molecules not flavonoids (Table 4).

In this present study, PCA was used to identify the variation in the antioxidant capacity of the plant extracts and to demonstrate how the eight parameters, namely, DPPH, ABTS, CUPRAC, FRAP, PPM, total phenolic content, total flavonoid content, and total tannins content contribute to the overall antioxidant capacity of *Cleome arabica*.

In this manuscript, PCA was applied to the data set of 11 different extracts to separate the samples according to similarity of the antioxidant activity. This technique is very useful because it reduces original variables based on principal component and gives the relationship between analyzed extracts and applied methods. The results obtained for each method were adopted as columns and the extracts as rows.

First, principal component analysis (PCA) applied to the data explained 75.25% of the total variance in the antioxidant profile of the *Cleome arabica* extracts. Moreover, the use of this unsupervised classification method often permits a simple representation of different sample data and their correlations. Two principal components were extracted because they have eigenvalues higher than 1.0, as suggested by the Kaiser criterion [49]. Eigenvalues give the measure of the variance accounted by the corresponding eigenvectors (components) [50]. Principal component 1 (PC1) explained up to 41.83% of total variance and PC2 explained 33.41% (Figs 1, 2).

ABTS, FRAP, CUPRAC, FRAP, and PPM are strongly positive correlated and grouped on the right side of the plot (Fig. 2), whereas DPPH is found in opposition side which confirmed no significant correlation with other assays. The extract N4 is on the negative side of the plot from all others because it has the highest negative loadings on PC1 (−1.45).

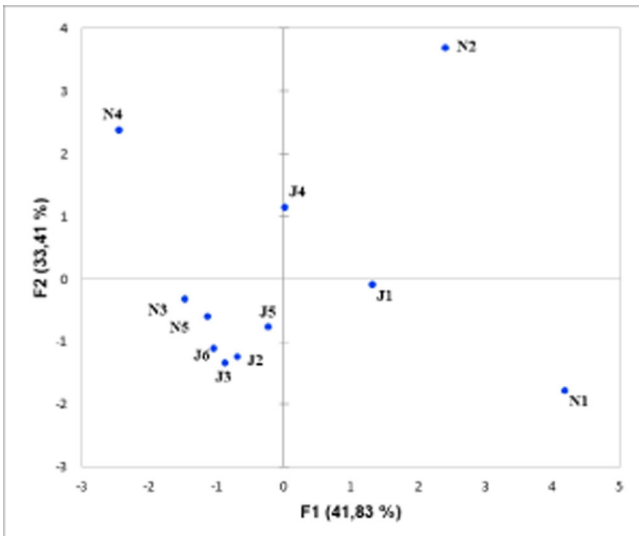

Fig. 1 Score plot obtained from PCA of the antioxidant capacities and the phenolic, flavonoid, and tannin contents of 11 *Cleome arabica* extracts

Table 4 Coefficients for the correlation between antioxidant capacities measured by ABTS, DPPH, PM, FRAP, and CUPRAC assays, total phenolic and flavonoid contents of *Cleome arabica* L. extracts

	Phenolics	Flavonoids	ABTS	FRAP	CUPRAC	DPPH	Molybdate
Phenolics	1	0.781	0.377	0.782	0.516	0.086	0.459
Flavonoids	0.781	1	0.0496	0.64	0.057	−0.399	0.048
ABTS	0.377	0.0496	1	0.629	0.9477	0.413	0.96
FRAP	0.782	0.646	0.629	1	0.692	0.075	0.713
CUPRAC	0.516	0.057	0.948	0.691	1	0.516	0.985
DPPH	0.0858	−0.399	0.413	0.075	0.516	1	0.521
Phosphomolybdenum	0.459	0.0478	0.966	0.713	0.985	0.521	1

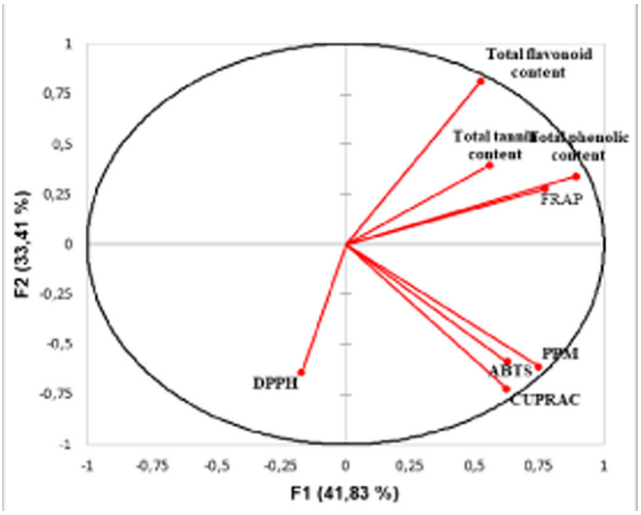

Fig. 2 Loading plot obtained from PCA of five antioxidant tests of 11 *Cleome arabica* extracts

It is expected because this extract was proved to have the highest antioxidant activity and therefore stands out from other extracts. In contrast, extract N1 has the highest negative loadings on PC2 (−1.78), and it is anticipated because this extract was proved to have the lowest antioxidant activity compared to other extracts. In contrast, a greater cluster of total phenolic, flavonoid, and tannins content with FRAP assay activity may account for their comparable activity due to their positional grouping at the same region of the plot (Fig. 2).

Conclusion

Up to date, the scientific documentation regarding antioxidant activity of plants extracts has been reported. Subsequently, we have explored in our study the antioxidant capacities of 11 extracts of local *Cleome arabica* leaves by utilizing five different in vitro assays. The present investigation provides useful information on antioxidant effects of these plant extracts, and the ethyl acetate fraction extract was showed maximum antioxidant power of all in vitro assays in comparison to the other extracts and standards.

Consequently, the present study provides scientific proof for traditional claim of Cleome arabica leaves as antioxidants. So, further in vivo studies are required to support the ethnomedicinal claim. Therefore, the present investigation will be supportive to the scientific documentation-related in vitro studies. Correlation between in vitro and in vivo studies may be helpful to understand the molecular mechanism of antioxidant process and to reveal phytochemicals of the extract responsible for this activity. Further studies need isolation and purification of active phytoconstituents with potent antioxidant activity.

Conflicts of interests the authors have no conflicts of interests to declare.

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