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Chemical Investigation of Phenolic Profiles and Antioxidant Activity of *Chrysanthemum Morifolium*

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Abstract

This study is focused on determining the phenolic profile and antioxidant activity of Chrysanthemum morifolium flowers and green aerial parts using methanol (MeOH) and ethyl acetate (EtOAc) solvents. The *in vitro* antioxidant activity was studied adopting three different tests; reducing power assay, Phosphomolybdenum antioxidative power assay, and inhibition of the free radical 1,1-di phenyl-2-picryl hydrazyl (DPPH). Total phenolic content (TPC); using Folin Ciocalteu reagent, ranged from 27.36±0.7 to105.72±0.64 mg gallic acid equivalent (mgGAE)/g D.W. While, total flavonoid content (TFC) ranged from 3.76±0.41 to 37.75±1.8 mg quercetin equivalent (mgQE)/g D.W. A total of 29 phenolics; including 15 phenolic acids and 14 flavonoids, were characterized and quantified using high performance liquid chromatography (HPLC). Ellagic acid was the prevalent phenolic in the green aerial parts MeOH extract and its EtOAc fraction (23.11and 35.63 mg/g, dry extract respectively). Hesperidin was the dominant flavonoid in the flowers MeOH extract amounted 43.23mg/g, dry extract. Flowers EtOAc fraction was the most efficient in all applied antioxidant methods. Green aerial parts methanolic extract was more dynamic than its EtOAc fraction, except in reducing power assay with EC50 value 2020.5±27.97 µg/mL. The results of this study implied that the analyzed samples were important natural sources for bioactive compounds with potential antioxidant activity.

Keywords: *Chrysanthemum morifolium*, Methanol extract, Ethyl acetate fraction, Phenolic composition, Antioxidant

1. Introduction

Reactive oxygen species (ROS) and free radicals are formed through physiological reactions in the body like respiration and are involved in many diseases [1]. A lot of fatal human diseases like cerebro–cardiovascular diseases, inflammation, and cancer have been recognized as a result of free radicals attack to protein, lipids, and nucleic acids [2].

Antioxidants protect the living system from oxidative stress and chronic diseases through oxygen scavengers, chelating metals, and free radicals capture. They can play a vital role in health care we're looking up [3]. Synthetic antioxidant compounds such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are responsible for harmful diseases. According to Lanigan and Yamarik [4] BHT had adverse effects on rats liver, lung and kidney because of its potential action as carcinogenesis. A further experimental study reported that both BHT and BHA had tumour-promoting activity [5]. Recent research suggests that naturally occurring antioxidant materials might be more suitable than synthetic compounds by humans [6].

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Plants are the most responsible for health benefits due to the accumulation of different phytochemicals such as tannins, flavonoids, phenolic acids, and their derivatives [7]. Regular consumption of phenolic acids affects health positively, diminishing the risk of neurodegenerative disorders and cancer [8]. In addition, many studies carried out on humans and animals cleared that flavonoids have multiple activities like antioxidant [9], anti–inflammatory [10], anti-viral, immunomodulatory activities against coronaviruses [11], antimicrobial, anticancer, and the prevention against metabolic diseases [12, 13].

Chrysanthemum morifolium is a popular ornamental plant, from the Asteraceae family, that is widely distributed throughout the world. Chrysanthemum is one of the most commercial cut flowers all over the world [14], and contains plenty of chemical compounds like apigenin, acacetin and chlorogenic acid [15,16]. A variety of flavonoid compounds such as kaempferol–glucuronide, luteolin–glucuronide, kaempferol–acetyl glucoside, luteolin–acetyl glucoside and quercetin–glucoside were found in C. morifolium [17].

Flavonoids and caffeoylquinic acids were the major bioactive compounds in C. morifolium [18]. A previous study carried out on 70% ethanol extract of C. morifolium stems revealed the presence of four new compounds: morineoliganosides A, B, C and heterophyllol –1–O–β–D – glucopyranoside [19]. It was clear that some members of genus Chrysanthemum had many different biological activities such as cardio vascular protective and anti–inflammatory [20], antioxidant [21], improve eyesight [22], reduce liver hyperactivity [23], and insecticidal activity [24].

The goal of the current study was to manipulate C. morifolium using different solvents to discover the phenolic profile and clarify its relationship with antioxidant properties.

2. Materials and Methods

2.1 Chemicals and Reagents

2.1.1. Chemicals

1. Aluminium chloride hexahydrate ($AlCl_3.6H_2O$), Ferric chloride ($FeCl_3$), Ascorbic acid, Trichloroacetic acid (CCl_3COOH), Potassium acetate (CH_3COOK), Potassium ferricyanide ($K_3[Fe(CN)_6]$), Disodium orthophosphate (Na_2HPO_4), Monosodium orthophosphate (NaH_2PO_4), Ammonium molybdate $(NH_4)_2MoO_4$, Methanol (MeOH), Ethyl acetate (EtOAc), Sulfuric acid ($Conc.H_2SO_4$), Sodium carbonate (Na_2CO_3), and Butylated hydroxytoluene (BHT) were purchased from El-Nasr Company for pharmaceutical chemicals.

2. Gallic acid, Quercetin dihydrate, 1,1-di phenyl-2-picryl hydrazyl (DPPH) radical were purchased from Sigma – Aldrich company.

2.1.2. Reagents

1. Folin - Ciocalteu′s reagent was purchased from Fluka, Biochemika.

2.2. Samples

In June 2016, *C. morifolium* with white flowers were obtained from a local market in Mansoura city, Egypt. Plant identification was carried out by specialist member, associate professor Mahmoud M. Kassem, Dept. of Floriculture, Fac. of Agriculture, Mansoura University, Mansoura, Egypt. A herbarium voucher specimen (CH-250) was deposited in Dept. of Horticulture, Fac. of Agriculture, Mansoura University.

C. morifolium was gently divided into two parts flowers and green aerial parts (leaves and stems), each part was cleaned thoroughly from earthy

pollutants and dust, later cut into small pieces and air dried in shade for two weeks. The air dried samples were ground separately into a fine powder and stored in polyethylene pouches until usage.

2.3. Extracts preparation

Methanol extraction of *C. morifolium* 1 kg of flowers and 2 kg of green aerial parts (leaves and stems) was done by maceration for 24 h at room temperature (4x 5L, for flowers and 4x8L, for green aerial parts). The combined extracts were evaporated using rotary evaporator at 45ºC to obtain crude methanolic extracts (Me extracts). 50 g of crude Me extract was redissolved in MeOH and water (200 mL, 1:4, v/v) and partitioned using EtOAc (400mLx4) for each sample (EAF).

2.4. Quantitative estimation of phytochemical compounds

2.4.1. Total phenolic content

Total phenolic content (TPC) was estimated using Folin–Ciocalteu reagent as mentioned in Limmongkon et al. [25]. The reaction mixture contained 0.5mL of Me extracts and their derived EAF, 0.1mL Folin-Ciocalteu reagent and 0.5 mL of 7.5% Na2CO3 solution. After incubation at room temperature in the dark for an hour, the absorbance was measured at 740 nm. The obtained results were noted as mg gallic acid equivalent (mgGAE/g), using gallic acid standard curve equation: (y = 0.0248x + 0.0591, R2 = 0.9979).

2.4.2. Total flavonoid content

Aluminium chloride colorimetric method illustrated in Munhoz et al. [26] was applied for total flavonoid content (TFC) determination with some modifications. Briefly, 2mL MeOH, 0.2mL of 1M CH_3COOK, 0.3mL of 10% $AlCl_3.6H_2O$ and 2mL of distilled water were added to 0.5mL of tested sample. After incubation at room temperature for 30 min, the absorbance was measured at 430 nm. Flavonoid content was calculated from the following equation: (y= 0.0046 xs+0.0585, R2=0.9995) using quercetin dihydrate as a reference and results were expressed as mg quercetin equivalent (mgQE)/g D.W.

2.4.3. Phytochemical profile identification using HPLC

Qualitative and quantitative analysis of phenolic and flavonoid compounds was carried out in Food technology research institute, Giza, Egypt. Flavonoid compounds were identified using the method mentioned in Mattila et al. [27], while phenolic compounds were identified using Goupy et al. [28] procedure.

Chromatographic analyses were performed on Agilent 1200 (Agilent, Germany) Series HPLC system equipped with a quaternary pump and an auto-sampler. The separation was executed on BDS 5 C18 (250×4.60 mm) packed stainless-steel column. The mobile phase systems were water/acetic acid (98:2, v/v) [A] and methanol/acetonitrile (50:50,v/v) [B], starting with 5% B with increasing B to levels of 30% at 25 min, 40% at 30 min, 52 % at 40 min,70% at 50 min and 100% at 55 min. The column temperature was arranged to 35ºC and the injection volume was 20µL. Detection wavelengths were set at 330 and 280 nm for flavonoid and phenolic compounds, respectively. All detected compounds were identified by comparing their retention time to known previously injected standards. The results are presented as milligrams per gram of dry extract (mg/g).

2.5. Antioxidant activity

2.5.1. Reducing power assay

Ferric reducing potential was recorded for the studied samples using the method proposed in Debnath et al. [29] which is based on the chemical reactions to convert Fe^{3+} to Fe^{2+} ion. Briefly, 1 mL of each tested sample was mixed with 2.5 mL of sodium phosphate buffer (0.2mM, 6.6pH) and 2.5 mL of 1% K3[Fe $(CN)_6$]. After incubation at 50° C for 20 min, 2.5mL of 10% CCl_3COOH were added to stop the reaction. 2.5mL of the reaction mixture were mixed with an equal volume of distilled water and 1mL of freshly prepared $FeCl_3$ 0.1% was added and left to incubate for another 10 min at room temperature. Absorbance was measured at 700 nm. Higher absorbance values confirm higher reducing power. Ascorbic acid was used as the reference standard.

2.5.2. Phosphomolybdenum antioxidative power assay

Total antioxidant capacity was estimated using phosphomolybdenum antioxidative power assay with slight modification [30, 31]. Exactly, 0.3 mL sample at a concentration of 500 µg/mL was mixed with 1 mL of the reaction mixture (28mM Na_2HPO_4, 4mM $(NH_4)_2MoO_4$ and 0.6mM H_2SO_4). The tubes were covered with aluminium foil and heated for 90 min in a hot water bath. After cooling at room temperature, the absorbance was measured at 765 nm. Ascorbic acid was employed for evaluation from the following equation: ($y = 0.0036x – 0.1417$, $R^2 = 0.991$).

2.5.3. DPPH radical scavenging assay

DPPH radical was applied to evaluate the antiradical activity depending on the rate of hydrogen atom abstracting by free radical and color reduction from deep violet to yellow. DPPH assay was performed following the procedure described in Dasgupta et al. [32] with total modification. Briefly, each sample with a fixed volume of 0.3 mL at different concentrations was mixed with 0.9 mL DPPH solution (60 µM in methanol). After incubation for 30 min, out of any luminance the absorbance was checked at 517 nm. Antioxidant activity was compared with those of standard BHT.

Scavenging activity percentage value was calculated as follows:

$$\frac{\text{Control} - \text{Sample}}{\text{Control}} x\ 100$$

Where, the control is the absorbance of DPPH and MeOH instead of the sample. Sample is the absorbance of DPPH and investigated samples.

Statistical analysis

This research was conducted on the basis of a completely randomized design (CRD) with three replications. Data was subjected to analysis of variance (ANOVA) and expressed as mean ± standard deviation using CoStat Ver. 6.400 statistical data analytical software. The comparison of mean values was performed using Duncan's multiple range test (DMRT) at $P<0.05$. Pearson correlation was used to evaluate the relationship between phytochemical compounds and antioxidant assays.

3. Results and Discussion

3.1. Quantitative estimation of phytochemical compounds

In the present work, TPC of C. morifolium Me extracts and their EAF varied according to the plant part and solvent polarity as seen in Table1. The highest TPC was found in the flowers EAF (105.72±0.64 mgGAE/g D.W) and the lowest was recorded for the green aerial parts Me extract (16.85±1.4mg GAE/g D.W). A converse trend was observed in TFC. The highest TFC was found in the flowers Me extract with a value of 37.75±1.8 mgQE/g D.W and the lowest was detected in the

green aerial parts EAF with a value of 3.76±0.41 mgQE/g D.W. Ethyl acetate fractions TPC was 2.9 and 1.5 fold higher than those in crude Me extracts for flowers and green aerial parts, respectively. On the contrary, TFC in ethyl acetate fractions was lower than those found in Me extracts, confirms that EAF concentrated more phenolic compounds than flavonoids. Generally, the composition and purity of phenolic compounds obtained from plant materials is affected by factors such as extraction method, sample size and the existence of interfering substances [33].

The lowest TFC in C. morifolium EAF was in agreement with previous study carried on Matricari pubescens by Metrouh-Amir et al. [34]. They reported that the lowest TFC was determined in the semi-polar solvent acetone, which suggests that the plant contains more heteroside flavonoids than aglycons.

This finding is consistent with a previous study demonstrated TPC and TFC in C. morifolium flower. Their values were 40.7±0.43 mgGAE/g and 31.95±0.44 mgQE/g, respectively [35]. Comparison with literature showed that the represented results in Table 1 were lower than those mentioned by Kennouche et al. [36], as they reported that TPC and TFC in EAF from Algerian C. segetum L. flower were 216.18±12.97mgGAE/g and 126.64±11.35 mgQE/g, respectively.

Table1

Total phenolic and flavonoid contents in *C. morifolium*

Sample	TPC (mg GAE/g D.W)	TFC (mgQE/g D.W)
Flowers Me extract	36.5 ± 0.23b	37.75 ± 1.8a
Flowers EAF	105.72 ± 0.64a	34.82 ± 0.64b
Green aerial parts Me extract	16.85 ± 1.4b	5.73 ± 1.27c
Green aerial parts EAF	27.36 ± 0.7b	3.76 ± 0.41c

Values in the same column followed by different uppercase letter were significantly different ($p< 0.05$)
Values represented the means ±SD (n=3)

3.2. Phytochemical profile using HPLC

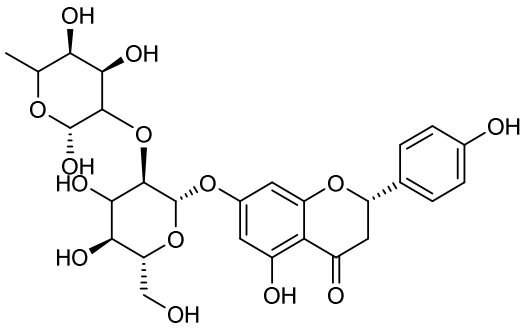
C. morifolium samples were subjected to identify their flavonoid and phenolic compounds, (Table 2 and Figures 1, 2). It is worthy to identify 14 flavonoid and 15 phenolic compounds. The majority of the identified flavonoid compounds were flavones. However, the dominant flavonoid compound was hesperidin (flavanone) amounted 43.23 and 23.58mg/g found in flowers and green aerial parts Me extracts, consecutively. Naringin was the second flavonoid compound amounted 35.19 mg/g detected in flowers Me extract, while it wasn't found in the green aerial parts Me extract. Naringenin was the lowest compound in amount 0.016 mg/g among all identified flavonoid compounds existed in flowers EAF. Phenolic acids are divided into two main classes: hydroxybenzoic acids (HBA) and hydroxycinnamic acids (HCA). The most predominant phenolic acid was ellagic acid (HBA) in green aerial parts Me extract and its EAF with a ratio of 23.11 and 35.63 mg/g, respectively. However, it was not found in flowers Me extract. The most widely characterized phenolic acids were 3,4,5tri-methoxycinnamic, α-coumaric, chlorogenic and salicylic acids. Gallic acid was the lowest one in amount (0.009 mg/g) found in green aerial parts Me extract. Chen et al. [35] reported the presence of quercetin, rutin, quercitrin, protocatechuic acid and vanillic acid in C. morifolium flower, whereas ferulic acid and p-coumaric acid weren't found. Apigenin, acacetin, caffeic acid and chlorogenic acid were mentioned in previous literature [15,16] in *C.*

morifolium flowers. Furthermore, apigenin, kaempferol and quercitrin were detected in HPLC chromatograms of Chinese C. morifolium Ramat flower [37]. As well, chlorogenic acid was detected in HPLC chromatogram of C. morifolium leaves EAF [38]. Miyazawa & Hisama [39] studied different EAF of C. morifolium flowers and results cleared the presence of apigenin and quercetin, which supporting the present study. Various C-glycosylated flavones were isolated from Chrysanthemum flowers, apigenin-8-C-hexoside-7-O-pentoside [40]. 6,8-C,C-diglucosylapigenin, 6-C-arabinosyl-8-C-glucosylapigenin and 6-C-xylosyl-8-C-glucosylapigenin[15]. As reported in earlier studies, the identified compounds had multiple biological activities such as: lowering blood pressure, cardio protective, hepatoprotective, liver protection, antioxidant, antibacterial, anti-inflammatory, anti-aging, anti-hyperglycemic, anticancer, antiviral and enhance plant tolerance against biotic and abiotic stress [41-44]. As previously mentioned by many authors, the presence or absence of any phytochemical compound and the difference between their amounts might be regretted to many factors such as growing conditions, plant age at harvest, harvest season, the type of plant part, used solvent and the extraction method [45, 46].

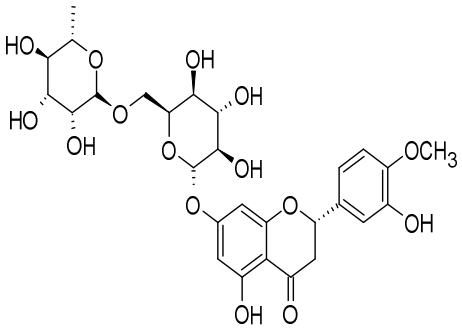
Table 2
The detected flavonoid and phenolic compounds in *C. morifolium* expressed as mg/g dry extract, Nd: not detected

Compounds	Flowers Me	Green aerial parts Me	Flowers EAF	Green aerial parts EAF	R_t
Flavones					
Apig-6-arbinose-8-glactose	0.38	0.14	0.019	0.93	11.6667
Apig-6-rhamnose-8-glucose	2.6	0.52	0.142	0.149	12.044
Apig-7-neohespiroside	1.56	0.55	0.021	1.1	13.05
Acacetin neo-rutinoside	4.8	Nd	0.086	0.198	15.06
Apigenin	3.34	0.18	0.095	0.088	16.566
Flavanones					
Naringin	35.19	Nd	0.37	0.74	12.347
Hesperidin	43.23	23.58	0.36	1.68	12.461
Naringenin	0.197	0.28	0.016	0.048	15.033
Hesperetin	1.38	7.14	0.02	0.037	15.341
Flavanol					
Epicatechin	0.64	0.05	0.11	0.195	9.619
Trihydric phenols					
Pyrogallol	2.41	1.29	0.5	0.73	6.925
Hydroxy benzoic acids					
Gallic	0.052	0.009	0.032	0.07	7.19
Protocatechuic acid	0.38	0.181	0.117	0.173	8.425
P-OH-benzoic	1.913	0.26	0.151	0.188	9.845
Vanillic	1.157	0.181	0.103	0.096	10.202
Ellagic	Nd	23.11	2.22	35.63	13.364
Salicylic	4.75	0.345	0.89	6.08	16.391
Hydroxy cinnamic acids					
Chlorogenic	7.04	0.46	0.172	0.41	10.103
Caffeic	0.11	0.036	0.03	0.04	10.21
P-coumaric	0.044	0.04	0.021	0.131	11.639

Ferulic	0.38	0.086	0.043	0.12	11.839
Isoferulic	Nd	0.074	0.138	0.51	12.215
Alpha-coumaric	11.7	0.195	0.017	0.038	13.266
3,4,5tri-methoxycinnamic	19.78	0.68	0.18	0.278	14.183
Coumarin	0.194	0.073	Nd	Nd	14.443

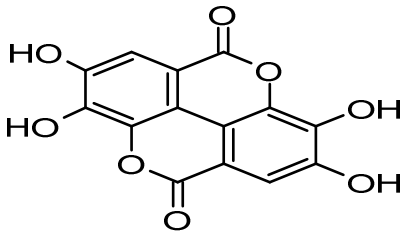


Naringin

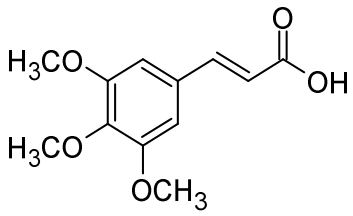


Hesperidin

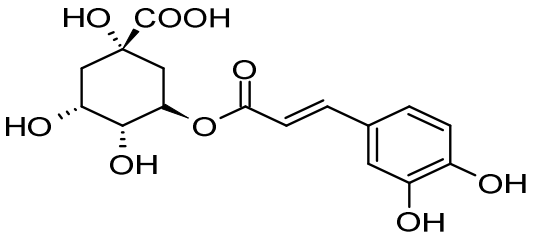
Fig.1. Structure flavonoid compounds



Ellagic acid



3,4, 5tri-methoxy cinnamic



Chlorogenic acid

Fig.2. Structure of phenolic acids

3.3. Antioxidant activity

3.3.1. Reducing power assay

This assay is used as an indicator for the potential antioxidant activity of the tested samples. The test solution color changes from yellow to different shades of blue or green due to the reduction of Fe^{3+} ions to Fe^{2+} [47]. The reducing ability of all Me extracts and their obtained EAF was expressed as

EC50 values as observed from Table 3. The EC50 values of all tested samples ranged from 25.16±0.02 to 2020.5±27.97 µg/mL. Flowers EAF showed the highest reducing power with EC50 value of 25.16±0.02 µg/mL superior on all tested samples, even the standard sample ascorbic acid, which recorded EC_{50} value of 191.78 ± 1.67. On the contrary, green aerial parts Me extracts was the weakest in reducing power with the highest EC_{50} value 2020.5±27.97µg/mL. The deficiency in reducing power might be due to the presence of hydrophilic phenolic compounds in small amounts which are easily accessible in Fe^{3+} reduction [48] like gallic, ferulic, caffeic and p-coumaric acids which were found in small amounts in green aerial parts Me extracts in comparison with those found in its EAF. Flowers Me extract EC_{50} value appeared opposite tendency to those [49], who stated that the EC50 value for C. indicum flower ethanolic extract was 1.31± 0.05 mg/mL.

Table 3
Antioxidant activity of *C. morifolium* using different solvents

Samples	Reducing power	Phosphomolybdenum	DPPH
Flowers Me extract	727.67 ± 29.75^{c}	200.2 ± 2.5^{b}	569.16 ± 16.09^{ab}
Flowers EAF	25.16 ± 0.02^{d}	288.52 ± 1.38^{a}	84.34 ± 1.24^{b}
Green aerial parts Me extract	2020.5 ± 27.97^{a}	237.06 ± 3.38^{ab}	212.3 ± 6.63^{ab}
Green aerial parts EAF	1090.5 ± 33.48^{b}	226.88 ± 2.49^{b}	701.86 ± 13.73^{a}
BHT	-	-	144.74 ± 8.78
Ascorbic acid	191.78 ± 1.67	-	-

Reducing power assay, expressed as EC_{50} (µg/mL), effective concentration achieved absorbance 0.5
Phosphomolybdenum antioxidative power assay, expressed as mg ascorbic acid equivalent per g extract (mgAAE/g)
DPPH assay, expressed as IC_{50} (µg/mL) inhibitory concentration which inhibit50% of the radical
Values in the same column followed by different uppercase letter are significantly different ($p<0.05$)
Values represented the means ± SD (n=3)

3.3.2. Phosphomolybdenum antioxidative power assay

The antioxidant capacity was evaluated through the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green phosphate Mo (V) complex at acidic pH. Increasing in absorbance values indicates higher antioxidant capacity. The values of phosphomolybdenum antioxidative power assay were estimated as mg ascorbic acid equivalent per g extract (mgAAE/g) and shown in Table 3. Flowers EAF had the highest total antioxidant capacity (288.52±1.38 mgAAE/g extract) between all analyzed samples. Nevertheless, flowers Me extract exhibited the lowest antioxidant capacity (200.2±2.5 mgAAE/g extract). Green aerial parts results were very close to each other 237.06±3.38 and 226.88±2.49 mgAAE/g extract for methanolic extract and its EAF, consecutively.

3.3.3. DPPH scavenging assay

This assay is a rapid, simple and vastly used method to measure the ability of compounds to act as free radical scavengers or hydrogen donors. After abstracting hydrogen from corresponding donor, the radical losses its characteristic deep violet color and turns to yellow. The IC_{50} values were used to compare the antiradical scavenging activity of different analyzed samples with those of standard antioxidant compound BHT. The IC_{50} values cleared that flowers EAF exhibited the highest antiradical scavenging activity with the lowest IC_{50} value

(84.34±1.24 µg/mL), while the external standard recorded 144.74 ± 8.78 µg/mL. Green aerial parts EAF had the lowest capacity to scavenge the radical with the highest IC50 value (701.86±13.73 µg/mL).

With regard to TFC and TPC in *C. morifolium* studied Me extracts and their EAF, the obtained results clarified that the highest TFC and TPC does not get along with the antioxidant activity. Flowers Me extracts had the highest TFC and green aerial parts EAF exhibited higher TPC compared with its methanolic extract, interestingly they did not appear a high antioxidant activity.

Flavonoids antioxidant activity is related to the structure and hydroxyl groups substitution. Sugar molecules substitution in identified flavonoid compounds could be responsible for their low antioxidant activity due to the spontaneous obstruction of sugar molecules, which increased blockage of the phenolic groups and decrease the accessibility of the membranes because of the large glycoside group [50]. In addition, the distribution of hydroxyl groups and their position determine the reactivity of flavonoid compounds [51]. The presence of hydroxyl groups at the R–3 position on the ring C gives flavonoid compounds excellent antioxidant capacity, while OH groups at the R–6 and R–7 on the ring A does not affect the ability to capture radicals. Glycosylation of the 3-OH group has highly suppressive effect on the antioxidant activity [52]. The ability to scavenge superoxide radical was significantly reduced after quercetin glycosylation [53]. Likewise, the power to reduce Fe^{3+} ions to Fe^{2+} [54]. Thus, the high content of rutin and quercitrin in flowers Me extract might be the reason for its low antioxidant activity. Phenolic acids antioxidant activity depends on the chemical structure, especially hydroxyl groups distribution [55]. Methoxy groups in phenolic acids decreased their antioxidant activity [56].Therefor, the high content of ferulic and isoferulic acids in green aerial parts EAF than its Me extract, might be a factor diminishing its antioxidant competence. Green aerial parts EAF contained higher amounts of caffeic and p–coumaric acids than its Me extract, which are known for their antioxidant capacity as mentioned in [57]. Nevertheless, it exhibited the lowest antioxidant activity among all investigated samples. The weakness in antiradical activity could be elucidated due to the antagonistic effect, which might happen inside each extract between main and minor components [58]. Herein, the antioxidant activity does not necessarily align with high amounts of phenolic compounds. Other non-phenolic compounds could be involved which have more potent antioxidant activities [59]. DPPH radical scavenging ability in C. indicum flower ethanolic and aqueous extracts was evaluated by Debnath et al. [49], their IC_{50} values were 240±0.02 and 270±0.01 µg/mL, respectively. Kennouche et al. [36] stated that the IC_{50} of Algerian C. segetum L. flowers EAF against DPPH radical was 23.58±1.71 µg/mL, which is nearly 4 fold higher than this finding. Another study estimated the potential antioxidant activity of *C. coronarium* leaves using DPPH radical cleared that Me extract was more efficient in radical scavenging than acetone extract [60], which was in accordance with the current work. The observed difference in antioxidant profile within each extract confirmed the fact that, no single antioxidant assay can completely explain the activities of phytochemical compounds as antioxidant agents. Thus, different antioxidant assays must be carried out in order to clear a complete antioxidant capacity profile [6, 61].

3.4. Correlation analysis between phytochemical compounds and antioxidant assays

The correlation coefficients between the mean values for TPC, TFC and antioxidant assays were analyzed positive correlation between TPC and TFC (r = 0.649, p<0.05). A strong significant negative correlation (r = - 0.585, p<0.01), (r = - 0.867, p < 0.001) was observed between TPC and DPPH assay and between TPC and reducing power assay, respectively. As well as, a strong positive correlation was found between TPC and phosphomolybdenum antioxidative power assay (r =0.807, p<0.01), which reflect the strong impact of phenolic compounds in *C. morifolium* antioxidant activity. Furthermore, strong negative correlation was noticed between TFC and reducing power assay(r = - 0.733, p< 0.01). However, a weak negative correlation was existed between TFC and DPPH assay (r = -0.242, p<0.05). Beside this, a weak positive correlation was found between TFC and phosphomolybdenum antioxidative power assay using person test and elucidated in (Table 4). The analysis of experimental data revealed a significant (r = 0.133, p< 0.05), indicating that flavonoids share strongly in reducing power assay of investigated samples. Whereas, flavonoid may not be the main dynamic compounds in phosphomolybdenum antioxidative power and DPPH assays. A previous study revealed a strong correlation between TFC and reducing power assay in five Me extracts from Cameroon spices [46], which support the present study. A lower r-value between TFC and phosphomolybdenum antioxidative power assay can be a result of long time exposition to a high temperature that might damage some sensitive phytochemical compounds [62]. There was a substantial correlation between TPC and antioxidant activity, and there was not any correlation between TFC and DPPH assay in colored soybean seeds [63].

Table 4
Correlation matrix between phytochemicals and antioxidant assays

	Phytochemicals		Antioxidant assays		
	TPC	TFC	DPPH	Phosphomolybdenum	Reducing power
TPC	1	-	-	-	-
TFC	0.649*	1	-	-	-
DPPH	- 0.585*	-0.242	1	-	-
PAP	0.807**	0.133	-0.787**	1	-
RP	- 0.867***	-0.773**	0.133	-0.444	1

* p< 0.05, significant correlation
**p< 0.01, very significant correlation
***p< 0.001, extremely significant correlation
(-) Previously mentioned values in the table

4. Conclusion

This study was the first trial to illustrate the phytochemical profile and antioxidant activity of C. morifolium EAF for both flowers and green aerial parts Me extract. The qualitative and quantitative analyses allowed the identification of phenolic and flavonoid compounds. The exploration of biological activities revealed that C. morifolium especially, flowers EAF and green aerial parts Me extract had excellent antioxidant activity. In view of the suggested results, it would be possible to invest C. morifolium as a source of natural constituents which could be integrated with pharmaceutical products.

5. Declaration of competing interest

The authors declare no conflict of interest.

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