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Chemical properties and antioxidant activity of essential oils of *Chrysanthemum morifolium* Ramat. and *Chrysanthemum indicum* L. in Vietnam

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Abstract In recent years, research into medicinal herbs with antioxidative activities has increased. Chrysanthemum morifolium and Chrysanthemum indicum are aromatic herb plants and that have long been used in traditional Vietnamese medicine. This study aims to evaluate the chemical compositions and antioxidative activities of essential oils hydrodistilled from the flower heads of C. morifolium and C. indicum. The chemical compositions of the essential oils were compared using gas chromatography/mass spectrometry (GC/MS) analysis. The antioxidative activity was determined and evaluated spectroscopically by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, metal chelating activity, reducing power, and total antioxidant capacity assays. According to the GC/MS results, chrysanthenone was predominant in the essential oils of both C. morifolium (64.14%) and C. indicum (32.02%). This is the first report of the identification of chrysanthenone as a major constituent of the essential oil of C. morifolium. Both Chrysanthemum oils were also revealed to possess antioxidant potential, exhibiting high antioxidative activities. In particular, the DPPH radical scavenging activities of the C.

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morifolium and *C. indicum* oils at a concentration of 100 mg/mL were 76.9 and 83.2%, respectively. The metal chelating values of *C. morifolium* and *C. indicum* were 0.85 and 0.76, whereas the reducing power values of that at 100 mg/mL were 0.76 and 0.71, respectively. This study provides the chemical properties of the essential oils of both *C. morifolium* and *C. indicum* grown in Vietnam and their potential antioxidant capacity.

Keywords Asteraceae · Chrysanthenone · Gas chromatography/ Mass spectrometry · Medicinal plants

Introduction

The genus *Chrysanthemum* L. (Family: Asteraceae) contains approximately 40 species that are distributed across countries in Asia (mainly China, Mongolia, and Japan) and Eastern Europe [1, 2]. Members of this genus are generally aromatic herbaceous (medicinal) plants, with *C. morifolium* and *C. indicum* being the most well-known among the existing species [2].

Aside from serving as ornamental plants because of their stunning flowers, the dried flower heads of *Chrysanthemum* species are commonly used as both traditional folk medicine and a popular tea, as they are believed to confer many benefits to human health [3, 4]. Zhu et al. [5] identified the major constituents in the essential oil of *C. indicum* flowers as 1,8-cineole, camphor, borneol, and bornyl acetate, which possess antihyperkinetic, antiasthmatic, antitussive, antimicrobial, antiviral, and parasiticidal activities. Similarly, camphor was confirmed as the major component of the essential oils from *C. indicum* and *C. morifolium* (accounting for 36.69% and 14.56%, respectively), exhibiting notable antitrypanosomal, antimicrobial, and antioxidative activities [4]. Furthermore, Yuan et al. [6] reported that polysaccharides

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extracted from different chrysanthemum teas (including *Coreopsis tinctoria*, *C. indicum*, and *C. morifolium*) exhibited remarkable antioxidative and antiglycation activities.

In addition to their antimicrobial and antioxidative effects, essential oils from Chrysanthemum are toxic to insects. According to Haouas et al. [7], the essential oils from three species of Chrysanthemum growing in Tunisia (C. coronarium, C. fuscatum, and C. grandiflorum) exhibited toxic and antifeedant effects against Tribolium confusum, one of the most prevalent pests in stored grains. The essential oil of C. grandiflorum was rich in sesquiterpenoids, whereas those of C. fuscatum and C. coronarium were rich in monoterpenoids [7]. Interestingly, the main common constituents of the essential oils from all three *Chrysanthemum* species were similar; namely, α -pinene, myrcene, α -humulene, β -caryophylene, spathulenol, and caryophyllene oxide [7]. In light of the different findings mentioned above, this study was conducted to investigate the chemical properties and antioxidant potential of C. morifolium and C. indicum grown in Hanoi, Vietnam.

Materials and Methods

Chemicals and reagents

2,2-Diphenyl-2-picrylhydrazyl hydrate (DPPH), ferrozine, ascorbic acid, quercetin, Folin-Ciocalteu reagent, potassium ferricyanide, sodium nitrate, aluminum chloride, dinitrosalicylic acid, ammonium molybdate, potassium ferricyanide, and trichloroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solvents of analytical grade were procured from Merck (Darmstadt, Germany). All other chemicals and reagents used were also of analytical grade.

Plant materials

Chrysanthemum morifolium Ramat. and *C. indicum* L. were cultivated in a field at the Ha Noi Research Center of Medicinal Plants (National Institute of Medicinal Materials, Ha Noi, Vietnam) in August 2021. The flower heads of *C. morifolium* Ramat. are white (Fig. 1A), whereas those of *C. indicum* L. are yellow (Fig. 1B). The flower heads (2.0 kg) were collected in December 2021.

Distillation of essential oils

The fresh flower heads (500 g) of *C. morifolium* and *C. indicum*, immediately after collection, were hydrodistilled separately using Clevenger's apparatus for 4 h. Each hydrodistillation was performed in triplicate. The essential oils obtained were separated and dehydrated by anhydrous sodium sulfate before being weighed and stored in a sealed glass vial at 4 °C until analysis.

Gas chromatography/Mass spectrometry analysis of the essential oils

For the gas chromatography/mass spectrometry (GC/MS) analysis, 10 μ L of the essential oil sample was diluted in 1 mL of *n*-hexane and mixed by vortexing. The homogeneous mixture was then analyzed using GC/MS on an Agilent apparatus (Capillary Column ZB-1 is Non-Polar GC Column/Specification; Length: 30.0 m, Film Thickness: 0.25 μ m, Diameter: 0.32 mm, Non-Polar Phase: 100% dimethyl polysiloxane). The sample was loaded into the injector at a split ratio of 30:1 at 180 °C. Helium was used as

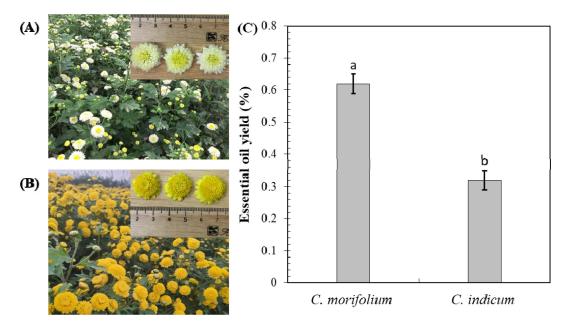


Fig. 1 Morphological characteristics of *Chrysanthemum morifolium* (A) and *Chrysanthemum indicum* (B) grown at the Ha Noi Research Center of Medicinal Plants (Ha Noi, Vietnam) and their essential oil yield (C)

the carrier gas at a flow rate of 1.61 mL/min, and the column pressure was set at 21.6 kPa (3.13 psi). The temperature program was as follows: initial temperature of 60 °C for 3 min, then increase to 120 °C at 4 °C/min and to 140 °C at 5 °C/min for 4 min, and finally increase to 180 °C at 10 °C/min hold for 2 min. For the MS analysis, an electron ionization source set at 280 °C and an electron ionization source set at 230 °C were used to isolate and fragment the ions. The GC program was set for 35 min, starting from 3 min and ending at 35 min (Solvent cut time-The filament delay is 2 min). GCMS-QP2010 Scan Mode with MS range was from 40 m/z to 350 m/z. The record peak is total ion chromatography (TIC). The GC operation conditions were identical to those described above for GC.

Identifying retention index values

Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes and by comparison of their mass spectral fragmentation patterns with those reported in the databases [8-10]. Quantification was done by the external standard method. Calibration curves of representative compounds from each class were drawn and used for quantification.

Antioxidant activity of the essential oils

The scavenging activity of the samples for the stable DPPH radical was determined using spectrophotometry. The DPPH radical is reduced when it reacts with an antioxidant capable of donating hydrogen. Changes in color (from deep violet to light yellow) were measured at 517 nm using an ultraviolet-visible spectrophotometer (CE 7500, Cecil Instruments Ltd, Cambridge, UK). The DPPH radical scavenging activity of the samples was measured according to the methods of Miliauskas et al. [11]. In brief, 0.1 mL of each sample at various concentrations was mixed with 1 mL of 0.2 mM DPPH dissolved in 0.004% methanol. The reaction mixture was incubated for 15 min at ambient temperature in the dark. A further mixture containing all the reagents without the sample served as a blank. After 15 min, the absorbance of the reaction mixture was measured at 517 nm with a spectrophotometer, and the DPPH radical scavenging activity of the sample was then calculated using the following formula: Radical scavenging activity $(\%) = [(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the blank and A_1 is the absorbance of the reacted sample. Ascorbic acid was used as a positive control. IC₅₀ values of the DPPH radical scavenging activity were also calculated.

The chelation of ferrous ions by the essential oils and standard was estimated using the method of Denis et al. [12]. In brief, 1 mL of a methanolic solution of the sample at various concentrations was added to 50 μ L of a 1 mM FeCl₂ solution. The reaction was initiated by the addition of 100 μ L of 1 mM ferrozine, whereupon the mixture was shaken vigorously and left to react at ambient temperature for 10 min. After the mixture had reached equilibrium, its absorbance was measured at 562 nm using a spectrophotometer

The reducing power of the samples was determined according to the method of Oyaizu [13], as described by Sidduraju et al. [14]. Different concentrations of each sample were separately mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide, and the mixture was then incubated at 50 °C for 20 min. Subsequently, 2.5 mL of 10% (w/v) trichloroacetic acid was added to the mixture, following which it was centrifuged at $3000 \times g$ for 20 min. Then, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) FeCl₃, and the absorbance was measured at 700 nm using a spectrophotometer. The increased absorbance of the reaction mixture indicates increased reducing power. The reducing power of ascorbic acid was also determined for comparison as a positive control.

The total antioxidant capacity of the samples was analyzed according to the method of Prieto et al. [15]. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate-Mo (V) complex at acidic pH. In brief, 0.1 mL of various concentrations of the sample was mixed with 1 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and then incubated at 95 °C for 90 min. Thereafter, the samples were cooled to 25 °C and the absorbance was measured at 695 nm against a blank (containing 1.0 mL of the reagent solution without the sample). The total antioxidant capacity was expressed as the absorbance of the sample, where a higher absorbance value indicates higher antioxidative activity. Ascorbic acid was used as a positive control for comparison.

Statistical analysis

Data were compared using Tukey's studentized range (honestly significant difference) test in Statistical Analysis System 9.1. Differences with a P value of less than 0.05 were considered statistically significant. All the assays were performed three times independently.

Results and Discussion

Essential oil yield

According to the GC-MS results, the yields of essential oils from *C. morifolium* and *C. indicum* flower heads were 0.62% and 0.32% (v/w), respectively, indicating that different *Chrysanthemum* species possess different amounts of essential oils (Fig. 1C). Youssef et al. [4] obtained essential oil yields of 0.18% (v/w) and 0.16% (v/w), respectively, from *C. morifolium* and *C. indicum* flower heads harvested at Heidelberg University (Heidelberg, Baden-Württemberg, Germany). These amounts are much lower than what it had been obtained from the same plant species grown

at the Ha Noi Research Center of Medicinal Plants. Indeed, other studies have noted that different varieties of *Chrysanthemum* differ in their essential oil yields and chemical compositions [6, 16]. Moreover, differences in the essential oil extraction methods used may also contribute to the differences in oil yield.

Chemical composition of the essential oils

The essential oils from *C. morifolium* and *C. indicum* flower heads were compared using GC-MS analysis, whereupon each oil was found to consist of 20 compounds (Tables 1, 2; Figs. 2, 3). This is again different from the study by Youssef et al. [4], which identified 64 and 55 compounds in *C. morifolium* and *C. indicum* essential oils, respectively.

In this study, the major constituent in the essential oil of *C. morifolium* was chrysanthenone (7; 64.14%), followed by camphor (9; 9.66%), thujone (8; 9.01%), gamma-eudesmol (19; 5.71%), and 1,8-cineole (4; 2.08%) (Table 1, Fig. 2). In *C. indicum*, chrysanthenone was also the major constituent (2; 32.02%),

followed by 4-epi-cubebol (9; 15.48%), beta-sesquiphellandrene (11; 6.19%), 9-Isopropyl-methyl-2-methyl-5-oxatrecyde (16; 6.05%), and shyobunol (17; 4.90%) (Table 2, Fig. 3). Similar to the yield results, the GC-MS analysis confirmed that the chemical compositions of C. morifolium and C. indicum essential oils were completely different from each other. Additionally, the predominant compounds identified in this study differ from those found in other studies. For example, Lawal et al. [17] indicated that the main constituent of the essential oil of C. morifolium flowers grown in Nigeria was cis-chrysanthenyl acetate (21.6%), followed by octadecanoic acid (19.5%) and borneol (15.5%). In another study, α -curcumene was found to be the most abundant volatile oil in C. indicum, accounting for 12.55% of all oil components [18]. Furthermore, camphor was found to be the major component of the essential oils from C. morifolium and C. indicum commercially obtained from China, accounting for 14.56 and 36.69%, respectively [4], whereas it accounted for 43.8% of the essential oil from C. indicum flowers grown in Korea [19]. However, in this study,

Table 1 Chemical compositions of the essential oils from flower heads of Chrysanthemum morifolium

| No. | Compounds | Retention Time(min) | Retention Index | | Contact (84) | | | | | | | |
|---|----------------------|---------------------|-------------------|------------------|---------------|--|---|--|-----|--|--|--|
| | | | RI _{Cal} | RI _{Db} | – Content (%) | | | | | | | |
| 1 | (E)-2-Hexen-1-ol | 3.263 | 861±5 | 864 | 0.33 | | | | | | | |
| 2 | Camphene | 5.223 | 940±5 | 937 | 0.29 | | | | | | | |
| 3 | Sabinene | 5.837 | 963±4 | 960 | 0.44 | | | | | | | |
| 4 | 1,8-cineole | 7.375 | 1018±5 | 1015 | 2.08 | | | | | | | |
| 5 | Pinene | 7.731 | 1029±5 | 1026 | 0.46 | | | | | | | |
| 6 | β-Ocimene | 8.069 | 1037±4 | 1036 | 0.33 | | | | | | | |
| 7 | Chrysanthenone | 9.468 | 1081±4 | 1079 | 64.14 | | | | | | | |
| 8 | Thujone | 9.827 | 1089±5 | 1091 | 9.01 | | | | | | | |
| 9 | Camphor | 10.499 | 1111±6 | 1111 | 9.66 | | | | | | | |
| 10 | Pinocarvone | 10.952 | 1125±4 | 1124 | 0.29 | | | | | | | |
| 11 | (-)-4-Terpineol | 11.97 | 1155±5 | 1154 | 0.68 | | | | | | | |
| 12 | Bornyl acetate | 15.622 | 1265±5 | 1262 | 0.57 | | | | | | | |
| 13 | β-caryophyllene | 20.553 | 1403±9 | 1401 | 0.4 | | | | | | | |
| 14 | (E)-β-Farnesene | 22.542 | 1448±3 | 1446 | 0.86 | | | | | | | |
| 15 | Cubebol | 23.778 | 1471±5 | 1474 | 0.7 | | | | | | | |
| 16 | 4-epi-Cubebol | 24.552 | 1489±3 | 1492 | 1.78 | | | | | | | |
| 17 | (+)-δ-Cadinene | 25.002 | 1506±5 | 1502 | 0.99 | | | | | | | |
| 18 | β-Sesquiphellandrene | 25.099 | 1505±5 | 1504 | 0.39 | | | | | | | |
| 19 | γ-Eudesmol | 28.794 | 1602±8 | 1598 | 5.71 | | | | | | | |
| 20 | 6-epi-Shyobunol | 31.179 | 1658±7 | 1661 | 0.89 | | | | | | | |
| Monoterpene (Sr. Nos. 2, 3, 5, 6) Monoterpenoids (Sr. Nos.1, 4, 7-11) Serquiterpene (Sr. Nos. 13, 14, 17, 18) | | | | | | | | | | | | |
| | | | | | | | Sesquiterpenoids (Sr. Nos.12, 15, 16, 19, 20) | | | | | |
| | | | | | | | | | 100 | | | |

- RI (Cal) (Standard Non-Polar): Retention Index with Standard Non-Polar GC Column in library spectra NIST 17 (Software NIST MS Search v2.3). Value Retention Index = Experimental R.I median ± Deviation

- RI (Database) (Column Rxi-1 MS): Retention Index from Database with Capillay Column Rxi-1 MS (Capillary Column Rxi-1 MS is Non-Polar GC Column/Specification: Length 30.0 m, Film Thickness: 0,25 μm, Diameter: 0.25 mm, Non-Polar Phase: 100% dimethyl polysiloxane)

| No. | Compounds | Retention Time (min) | Retention Index | | Contant (0/) | |
|---|--|-------------------------|-------------------|------------------|---------------|--|
| INO. | Compounds | | RI _{Cal} | RI _{Db} | - Content (%) | |
| 1 | (2S)-2-Oxiranyl-6-methyl-5-heptene-2-ol | 8.625 | 1050±5 | 1053 | 0.92 | |
| 2 | Chrysanthenone | 9.451 | 1080±5 | 1079 | 32.02 | |
| 3 | Epoxy-α-terphenyl acetate | 15.707 | 1263±3 | 1265 | 3.54 | |
| 4 | (R)-Lavandulyl acetate | 15.952 | 1273±2 | 1272 | 1.69 | |
| 5 | (E)-β-Farnesene | 22.551 | 1448±3 | 1446 | 1.19 | |
| 6 | β-Copaene | 23.241 | 1460±8 | 1462 | 1.82 | |
| 7 | Cubebol | 23.782 | 1470±5 | 1474 | 2.78 | |
| 8 | Zingiberene | 24.088 | 1484±4 | 1481 | 2.99 | |
| 9 | 4-epi-Cubebol | 24.557 | 1489±3 | 1492 | 15.48 | |
| 10 | (+)-δ-Cadinene | 25.007 | 1506±5 | 1502 | 2.3 | |
| 11 | β-Sesquiphellandrene | 25.110 | 1505±5 | 1505 | 6.19 | |
| 12 | Andrographolide | 26.709 | 1545±8 | 1545 | 3.77 | |
| 13 | Tricyclo[5.2.2.0(1,6)undecane-3-ol, 2- methylene-6,8,8-trimethyl- | 27.155 | 1555±5 | 1556 | 3.04 | |
| 14 | Isoaromadendrene epoxide | 27.558 | 1568±1 | 1567 | 2.49 | |
| 15 | Aromadendrene oxide-(2) | 30.106 | 1635±5 | 1633 | 3.09 | |
| 16 | 9-Isopropyl-methyl-2-methyl-5-oxatrecyde | 30.288 | 1640±5 | 1638 | 6.05 | |
| 17 | 6-epi-Shyobunol | 31.180 | 1659±3 | 1661 | 4.9 | |
| 18 | 7-Methyl-4-(1-methylethylidene)bicyclo[5.3.1]undec-1- en-8-ol | 31.578 | 1674±3 | 1672 | 3.44 | |
| 19 | 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol | 32.057 | 1686±5 | 1685 | 1.54 | |
| 20 | 8-Cedren-13-ol | 33.531 | 1740±5 | 1742 | 0.73 | |
| Monoterpenoids (Sr. Nos.1-4) | | | | | | |
| Serquiterpene (Sr. Nos.5, 6, 8, 10, 11) | | | | | | |
| Sesquiterpenoids (Sr. Nos.7, 9, 13-20) | | | | | | |
| Diterpenoids (Sr. Nos.12) | | | | | | |
| Total identified | | | | | | |

 Table 2 Chemical compositions of the essential oils from flower heads of Chrysanthemum indicum

- RI (Cal) (Standard Non-Polar): Retention Index with Standard Non-Polar GC Column in library spectra NIST 17 (Software NIST MS Search v2.3). Value Retention Index = Experimental R.I median ± Deviation

- RI (Database) (Column Rxi-1 MS): Retention Index from Database with Capillay Column Rxi-1 MS (Capillary Column Rxi-1 MS is Non-Polar GC Column/Specification: Length 30.0 m, Film Thickness: 0,25 μm, Diameter: 0.25 mm, Non-Polar Phase: 100% dimethyl polysiloxane)

camphor was identified as only the second most abundant component in C. morifolium, where it did not exceed 10%. Furthermore, we identified chrysanthenone as the predominant component of the essential oil of C. morifolium and C. indicum, accounting for a very high amount of 64.14 and 32.02%, respectively. Similarly, Chang et al. [20] reported that chrysanthenone was among the major aroma components of C. indicum, accounting for 10.01%, which is 3 and 6 times lower than the amount of chrysanthenone obtained from C. indicum and C. morifolium in this study. This greatly highlights that the C. indicum and C. morifolium grown in Ha Noi is a particularly good source of chrysanthenone. In addition, to the best of our knowledge, this is the first report of the identification of chrysanthenone as a constituent of the essential oil of C. morifolium, let alone it being the major compound. These findings greatly highlight the impact of geographical distribution on the chemical compositions of different Chrysanthemum species.

Antioxidative activity of the essential oils

Pharmacological studies have confirmed that Chrysanthemum oils possess diverse bioactivities, including antioxidative and antimicrobial effects [4,21]. Here, the antioxidant activity of the essential oils of C. indicum and C. morifolium was tested. The stable DPPH can be used to study the reaction kinetics of antioxidants, as well as to quantify and compare the free radical scavenging capacities of different antioxidants. In the present study, the DPPH radical scavenging activity of each essential oil was quantified as its percentage inhibition values, which were 15.2, 45.2, 63.3, and 83.2% at the concentrations of 0.1, 1.0, 10, and 100 mg/mL, respectively, for the C. morifolium samples (Fig. 4A). Similarly, the inhibition values for the C. indicum essential oil were 13.5, 42.6, 58.7, and 76.9% at the sample concentrations of 0.1, 1.0, 10, and 100 mg/mL, respectively (Fig. 4A), whereas those of the positive control (ascorbic acid) were 52.7, 92.7, 100, and 100% at the same respective concentrations (Fig. 4A). The DPPH radical

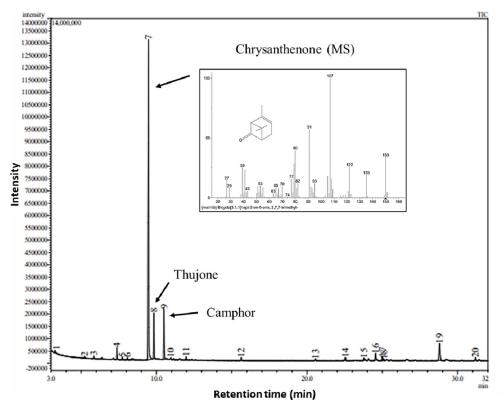


Fig. 2 Gas Chromatography/Mass Spectrometry profiles of the compounds in the essential oil obtained from flower heads of *Chrysanthemum morifolium*. The chemical structures of the main compounds are shown in the figure

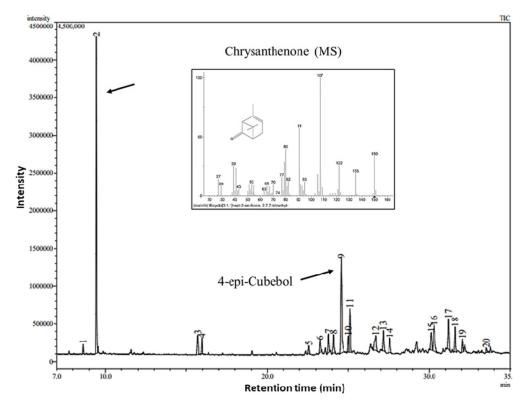


Fig. 3 Gas Chromatography/Mass Spectrometry profiles of the compounds in the essential oil obtained from flower heads of *Chrysanthemum indicum*. The chemical structures of the main compounds are shown in the figure

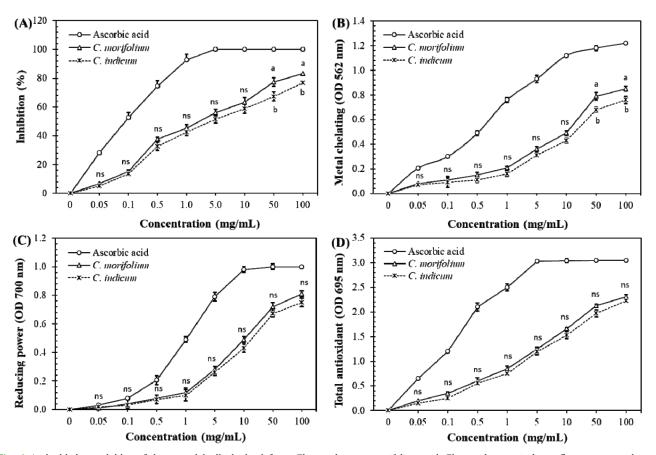


Fig. 4 Antioxidative activities of the essential oil obtained from *Chrysanthemum morifolium* and *Chrysanthemum indicum* flowers versus that of ascorbic acid as a reference drug in (A) 2,2-diphenyl-1-picrylhydrazyl, (B) metal chelating, (C) reducing power, and (D) total antioxidant capacity assays. Results are the means \pm SD of triplicate measurements. NS: no significant difference. Different letters above the standard error bars indicate a significant difference at each observation time based on Tukey's HSD test ($p \le 0.05$). ns: not significant

scavenging activities of the C. morifolium and C. indicum oils ranged from 76 to 83% at the concentration of 100 mg/mL. In addition, the calculated IC₅₀ values were 2.79 and 3.01 mg/mL for the C. morifolium and C. indicum oils, respectively, versus 0.092 mg/mL for ascorbic acid. Previously, Youssef et al. [4] had indicated that the essential oils from the flower heads of C. indicum and C. morifolium exhibited substantial antioxidant potential, with IC₅₀ values of 2.21 and 2.59 mg/mL, respectively, in the DPPH radical scavenging assay, 1.90 and 2.89 mg/mL, respectively, in the hydroxyl radical scavenging assay, and 4.40 and 5.92 mg/mL, respectively, in the superoxide radical scavenging assay. Additionally, Gong et al. (2019) [22] reported that their DPPH radical scavenging assay of 15 C. morifolium cv. 'Hangbaiju' plants grown in China revealed IC₅₀ values ranging from 1.69 to 3.04 mg/L. Taken together, our results indicate that the essential oils from the C. morifolium and C. indicum plants used in this study show remarkable antioxidant potential, which is in line with the findings of other studies demonstrating the antioxidative property of Chrysanthemum species [4,6,16].

According to recent reports, essential oils from the medicinal

plants play several biological roles, including antioxidative or free radical scavenging activities. Youssef et al. [4] identified camphor (14.56%), curcumene (10.50%), and τ -eudesmol (8.92%) to be the major constituents in *C. morifolium* and camphor (36.69%), isoborneol (7.64%), α -terpinene (5.73%), and caryophyllene oxide (5.46%) as those from *C. indicum*. Camphor and chrysanthenone are also the main components in the essential oils of *Achillea biebersteini* Afan. (Asteraceae), *Artemisia judaica* L., *Artemisia herba-alba* [23-25], which have been shown to have potential antioxidative activity. Similarly, this is also the case with the essential oil of *C. morifolium* in our study. However, despite that chrysanthenone was the main compound of the essential oils of *C. morifolium* and *C. indicum*, whether this compound possesses antioxidant activity has not been confirmed, thus requiring pure chrysanthenone to certify that assumption.

The antioxidative activities of *C. morifolium* and *C. indicum* were further determined through estimations of their metal chelating activity, reducing power, and total antioxidant capacity, where the increased absorbance of the reaction mixture indicates increased activity. The results showed that there were no differences

between the C. morifolium and C. indicum oils in terms of their metal chelating activity, reducing power, and total antioxidant capacity, albeit the results for C. morifolium always tended to be higher than those for C. indicum in all three assays (Figs. 4B, C, and D). The metal chelating activities of C. morifolium, C. indicum, and ascorbic acid were 0.49, 0.43, and 1.12, respectively, at 10 mg/mL sample concentration and 0.85, 0.76, and 1.22, respectively, at 100 mg/mL concentration (Fig. 4B). The reducing power values of 100 mg/mL C. morifolium, C. indicum, and ascorbic acid were 0.76, 0.71, and 1.00, respectively (Fig. 4C). For 50 mg/mL of sample, the reducing power values of the C. indicum and C. morifolium oils were 0.67 and 0.72, respectively, versus 1.00 for ascorbic acid. Similar to the reducing power, the total antioxidant capacity of 50 mg/mL of C. morifolium, C. indicum, and ascorbic acid reached values of 2.13, 1.97, and 3.05, respectively (Fig. 4D).

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Declaration of Competing Interest No potential conflict of interest was reported by the author(s).

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