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Ethno-pharmacological activity of the medicinal plant *Chrysanthemum dante purple*

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Abstract: Six compounds were extracted from the ethyl acetate fraction of *Chrysanthemum dante purple* maxim. flowers for the first time, and identified as; luteolin (**1**), quercetin (**2**), apigenin 7-*O*-*β*-D-glucopyranoside (**3**), luteolin 7-*O*-*β*-D-glucopyranoside (**4**), rutin (**5**) and daucosterol (**6**). The structures of the extracted compounds had been established by comparison of their NMR and MS data with published ones. Free radical scavenging activity of flowers compound (**1**), total methanolic extract and fractions and confirmed that they had potent antioxidant activity even with lower concentrations, IC_{50} of all tested samples was temperately low between 0.3 and 0.6 mg/ml. All samples showed also potent cholesterol reduction ability. Concerning anti-inflammatory activity, the protection provided by compound (**1),** (luteolin), and butanol fraction were the most potent even with the lowest concentration. Further anti-proliferative activity of all tested samples showed good anti-tumor activity even with low concentrations against human colorectal (HCT-116) and hepatic (HepG-2) cancer cells exceeding Doxorubicin and moderate antitumor activity against breast (MCF-7) cancer with no cytotoxic activity against BJ-1 human skin healthy cell-line. This could promote the use of *C. dante purple* flower extract for pharmacological and health purposes.

Keywords: *Chrysanthemum dante purple*, phytoconstituents, antioxidant, anti-cholesterol, anti-inflammatory, anti-proliferative activities.

1. INTRODUCTION

Natural and traditional therapies are crucial. Natural products and its derivatives are a source of therapeutic medicines and structural diversity [1]. Out of the world's 75,000 higher plant species, only 10% are employed in traditional medicine. Only 1 to 5% has been scientifically investigated and has therapeutic value [2]. Drug discovery using natural products is a challenging approach for designing new leads. It describes natural bioactive substances, their phytochemical examination, characterization, and pharmacological study. It focuses on the success of these resources in discovering new and effective medication molecules for humans. Natural materials have been used therapeutically for years. Through structural elucidation and identification, drug discovery research must produce robust and viable lead compounds. New technologies have transformed natural product screening for drug discovery. Using these tools allows us to test new molecules and establishing natural products as a primary source for drug development [3].

For thousands of years, traditional medicine had protected people from several diseases, According to the WHO, more than 80% of the world's population relies on traditional and folk medicine, almost all of them based on plant medicines. About 20% only of the world's plants had been tested for pharmacological or biological properties [4]. The "One Root of Medicine and Food" refers to the fact that many herbs used in traditional medicine are also

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consumed as food for health care and disease prevention [5]. For health care, medical cosmetology, and the adjuvant treatment of severe illnesses, flowers are a rich source of plant essential oil that has multiple bioactivities with unique efficacy and benefits [6]. It has been hypothesized that the phytochemicals found in flowers such as flavonoids (antioxidants), anthocyanins (antibacterial), alkaloids (anti-inflammatory), neuroprotective (anticancer), and phenolic acids (visceral injury prevention) are responsible for the health benefits attributed to their consumption [7].

All of these factors contributed to the growing acceptance of flowers as dietary herbal remedies in traditional Chinese medicine. In the Asteraceae family, there are over 1600 genera and 2500 species, but the majority of them are found in the subtropical dry and semi-arid regions [8]. Chicory, sunflower, lettuce, dahlias, and daisies are well-known species of Asteraceae family, having several medicinal properties [9]. Asteraceae species had a long history in traditional medicine and a wide range of therapeutic applications. For over 3000 years, some members of the family had been grown for culinary and medicinal purposes. Members of the Asteraceae family have anti-inflammatory, anti-microbial, antioxidant, and hepatoprotective properties [10].

Among the most common chemical compounds found in Asteraceae are flavonoids, coumarins, terpenoids, sesquiterpene lactones, poly acetylene-derived fatty acids, and polysaccharide fructans [11, 12]. When it comes to flowers, the name *Chrysanthemum* comes from the Greek words chrysos (gold) and anthemon (beautiful) (flower) [13], it is native to East Asia and northeastern Europe and the center of diversity is in China (https://en.wikipedia.org/wiki/Chrysanthemum).

Antimicrobial, antioxidant, anti-inflammatory, anti-genotoxic, and anticancer properties of *Chrysanthemum* species, as well as hepatoprotective, neuroprotective, and immune-regulating effects are primarily responsible for the medicinal properties of these plants [14, 15]. There are many varieties of *chrysanthemum*, which is one of the world's most valuable floricultural crops. It has anti-allergy, anti-inflammatory, anti-cancer, anti-obesity, immune regulation, hepatoprotective, and nephroprotective properties [16]. The current study was aimed to investigate new compounds from the total methanolic extract, hexane, butanol and ethyl acetate fractions of *C. dante purple* flowers as well as identification of their antioxidant, cholesterol reduction and anti-inflammatory activities of the extracts and isolated compounds.

2. METHODS

2.1. Plant materials

Fresh *Chrysanthemum dante purple* flowers (hybrid) were purchased in October 2018 from Mahmoud Helmy Garden, Giza, Egypt and were kindly identified by Mrs. Terase Labib, Taxonomist of Al-Orman Garden, Giza, Egypt. A voucher specimen {Code: A-5} had been kept in the Herbarium of Pharmacognosy department, Faculty of Pharmacy (Girls), Al-Azhar University.

2.2. General experimental procedures

^{1}H- and ^{13}C-NMR measurement, **ESI-MS** positive and negative ion acquisition modes, **Column Chromatography** and **TLC** were carried out according to manufacturer's instructions.

2.3. Extraction and isolation

The powder of *Chrysanthemum dante purple* flowers (1.2 kg) was exhaustively extracted with 70% aqueous methanol (3 × 8L) according to general instructions.

2.4. Spectral Data

Luteolin [1], Quercetin [2], Apigenin 7-*O*-*β*-D-glucopyranoside [3], Luteolin 7-*O*-*β*-D-glucopyranoside [4], Rutin [5] and β-sitosteryl-3-*O*-*β*-D-glucopyranoside (Daucosterol) [6] were identified using NMR spectroscopy.

2.5. DPPH free radical scavenging assay was performed according to [17].

2.6. In vitro cholesterol reduction assay was performed according to [18].

2.7. Anti-inflammatory assay was performed according to [19].

2.8. Anti-tumor activity was performed according to [20].

2.9. Statistical analysis

Means and standard deviations were calculated for each experiment and are shown in comparison to the control. Origin and SPSS are used for statistical analysis.

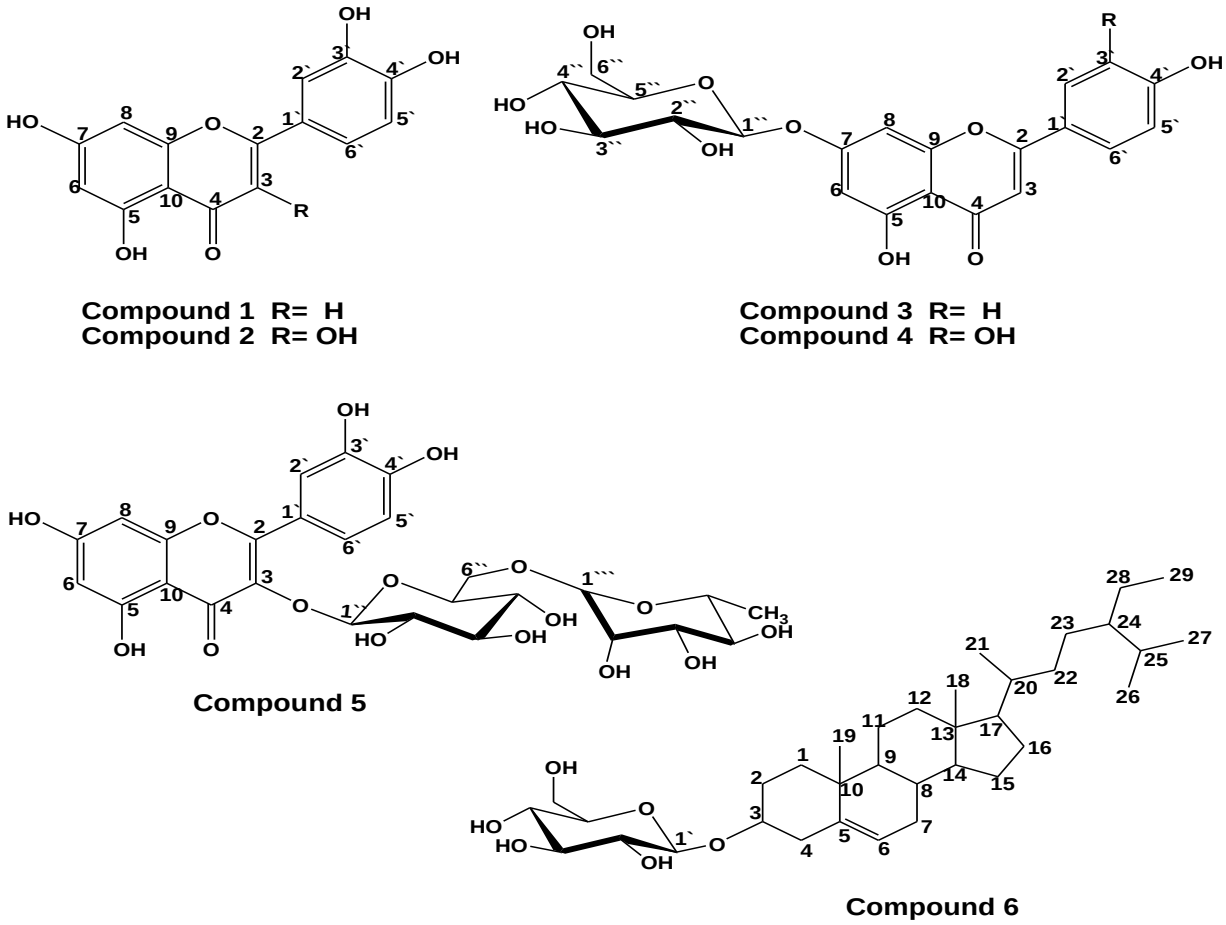

Figure 1. Structures of compounds (1-6)

3. RESULTS and DISCUSSION

3.1. Identification of the isolated compounds

Six compounds had been isolated from the ethyl acetate fraction of *Chrysanthemum dante purple* flowers for the first time, and were identified as luteolin **(1)**, quercetin **(2)**, apigenin 7-*O*-*β*-D-glucopyranoside **(3)**, luteolin 7-*O*-*β*-D-glucopyranoside **(4)**, rutin (**5)** and daucosterol **(6)**. Their structures were established by comparison of their NMR and MS data with published ones [21-24].

3.2. Antioxidant activity

The results of the antioxidant action of *C. dante purple* compound (**1),** (luteolin), total methanolic extract and fractions at different concentrations (0.33, 1.0, 1.33 and 1.67 mg/ml), estimated by the DPPH scavenging activity, were presented in Fig. 2. All the tested samples exhibited potent antioxidant activity ranged from 91.05±2.76 to 95.88±2.91 using 1.67 mg/ml, from 86.01±2.33 to 92.70±2.76 using 1.33 mg/ml, from 58.64±2.62 to 74.59±2.18 using 1.0 mg/ml, and from 41.26±2.76 to 47.74±2.04 using 0.33 mg/ml. Ascorbic acid (0.1%) was used as a positive control with 100% antioxidant activity at this concentration. The IC_{50} of all fractions was moderately low between 0.35 and 0.50 mg/ml. These results indicated that all extracts have potent antioxidant activity even with lower concentrations. Flavonoids found enormously in Asteraceae were reported to have potent antioxidant activity [25, 26].

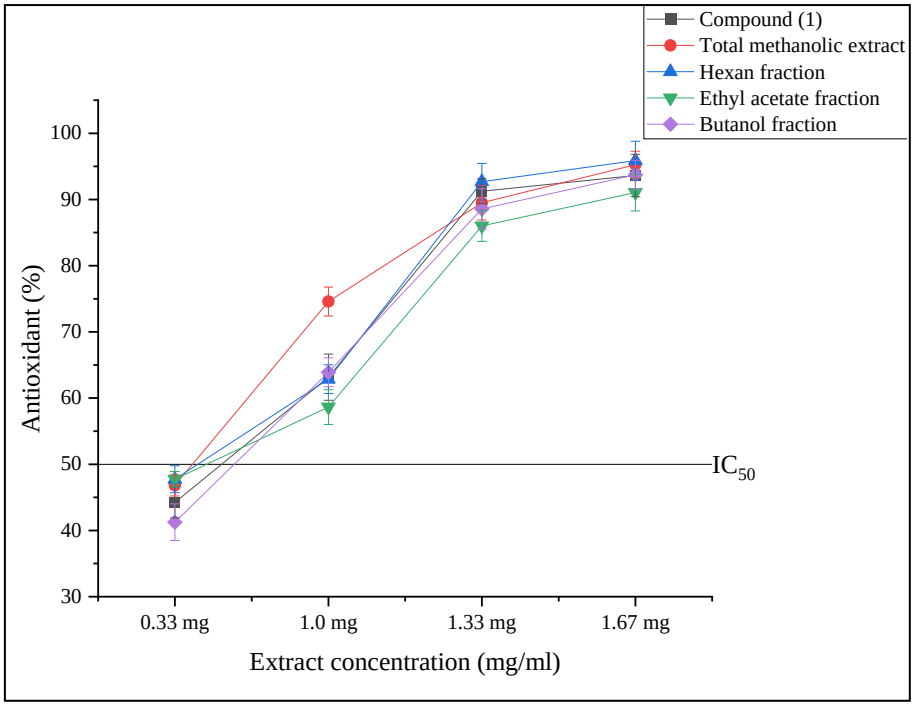

Figure 2. Antioxidant activity of *C. dante purple* flowers compound (1), total methanolic extract, and fractions showing IC_{50}

3.3. *In vitro* cholesterol reduction assay

In a preliminary investigation of *in vitro* ability of compound (**1**), total methanolic extracts, *and* fractions of *C. dante purple* flowers to break down cholesterol, all the tested samples showed potent cholesterol reduction ability ranged from 86.17±0.80 to 90.72±1.34 and from 88.64±1.07 to 99.05±0.27 after 48 and 96 hour of incubation, respectively. Interestingly, ethyl acetate fraction was approximately able to break down all the amount of cholesterol after 96 hour of incubation (up to 99%±0.27). Investigating the ability of flower fractions to lower cholesterol *in vitro* showed that all the fractions were able to degrade cholesterol with almost the same potencies regardless of time needed. Developing cholesterol-lowering natural compounds became of great interest due to the well-known severe side effects caused by cholesterol-lowering drugs, statins. The notable cholesterol-lowering potential of *C. dante purple* flowers fractions may be due to the existence of flavonoids and coumarins that could be the main cause of cholesterol break-down [25, 27].

3.4. Anti-inflammatory assay

The results of the assay as presented in Fig. 3 indicated that compound (**1),** plant total methanolic extract, and fractions have different anti-inflammatory properties comparable to conventional medicines. However, when the concentration of the extract is increased, protection from hemolysis improves, with the maximum level of protection being attained at a concentration of 0.001 mg / reaction mixture. The test indicated that protections provided by compound (**1)** and butanol fraction were the most potent even with the lowest concentration (96.32±0.24, 96.49±0.35 using 0.001 mg and 94.67±0.20, 94.77±0.17 using 0.0005 mg, respectively). The other samples (total methanolic extract, *n*-hexane and ethyl acetate extracts) exerted also excellent protection even with small concentrations suggesting that both minimal and maximal concentrations of samples are equally efficient in providing protection. Diclofenac sodium was used as a positive control with 87.85±1.06 activity at 0.001 mg.

Research on HRBCs' anti-inflammatory properties has relied on the HRBC membrane stabilization method, first proposed by Chou in 1997 [28] due to the close similarity between RBCs and lysosomes and the complex relationship between lysosome rupture and inflammation [29]. The aim of the study was to examine if floral extracts could stabilize HRBC membranes in a similar way to lysosomes. Flower extracts rich in flavonoids were the primary source of antioxidant and anti-inflammatory activity [30].

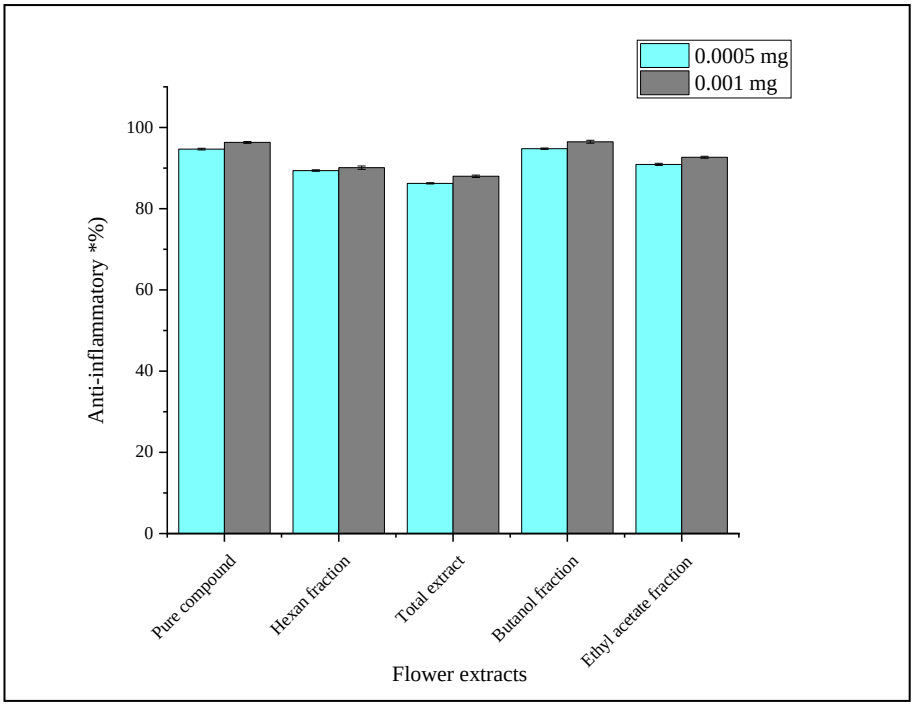

Figure 3. Anti-inflammatory activity of *C. dante purple* flowers compound (1), total methanolic extract and fractions

3.5. Anti-tumor activity

C. dante purple flowers compound (**1**), total methanolic extract and fractions were examined *in vitro* for anti-proliferative efficacy against human cancer cell lines HepG-2, MCF-7 and HCT-116 using the MTT assay versus one human healthy cell line (BJ-1). Tested substances were compared to Doxorubicin's activity against three cancer cell lines. In a dose-dependent way, all of the substances examined inhibited all the tested cancer cells (Fig. 4); the results showed that compound (**1**) and total methanolic extract, respectively have more potent cytotoxic activities, while *n*-hexane, ethyl acetate and butanol fractions have less but still good cytotoxic activity against the tested cell lines comparative to that of Doxorubicin. However, all tested compounds had less antitumor activity against MCF-7 human breast cancer cells relative to the other cell lines and the reference drug (Fig. 5).

In case of HepG-2, human liver cancer cells, all the tested samples had strong activity exceeding that of the control. Concerning HCT-116, human colon cancer cells, compound (**1**) and total extract exerted the highest antitumor activity exceeding Doxorubicin, while the other samples had comparable cytotoxic activity (Fig. 6). From the above results, it is concluded that compound (**1**) and total methanolic extract were selectively active against all the three tested human cancer types. All fractions and compound (**1**) showed good antitumor activity against HepG-2 liver cancer cells exceeded Doxorubicin even with low concentrations. All fractions were tested against non-tumor fibroblast-derived cell line (BJ) and demonstrated very low cytotoxicity. All fractions showed lower IC_{50} values than that of Duxorubicin in case of HepG-2 cell lines and moderate IC_{50}s with the other cancer cell-lines (Table 1).

The potent antitumor activity of flowers total methanolic extract and all fractions reported in this study against HepG-2, MCF-7, and HCT-116 human carcinomas using the MTT assay with almost no cytotoxic activity towards the human healthy cell line (BJ-1) is really of great interest. Using low concentrations of each extract could exert potent anti-proliferative activity against some tested cancer cell-lines which could exceed Doxorubicin in some cases. Some chemotherapeutic medicines cause apoptosis in target cells by generating free radicals; antioxidants, on the other hand, can scavenge these radical molecules and limit the side effects of therapeutic agents [31, 32]. Inhibiting antioxidant enzymes in this manner, on the other hand, could prevent cancers from proliferation.

On the whole, we've found that extract from *C. dante purple* flowers exhibits significant bio-therapeutic activity that could be utilized for therapeutic purposes.

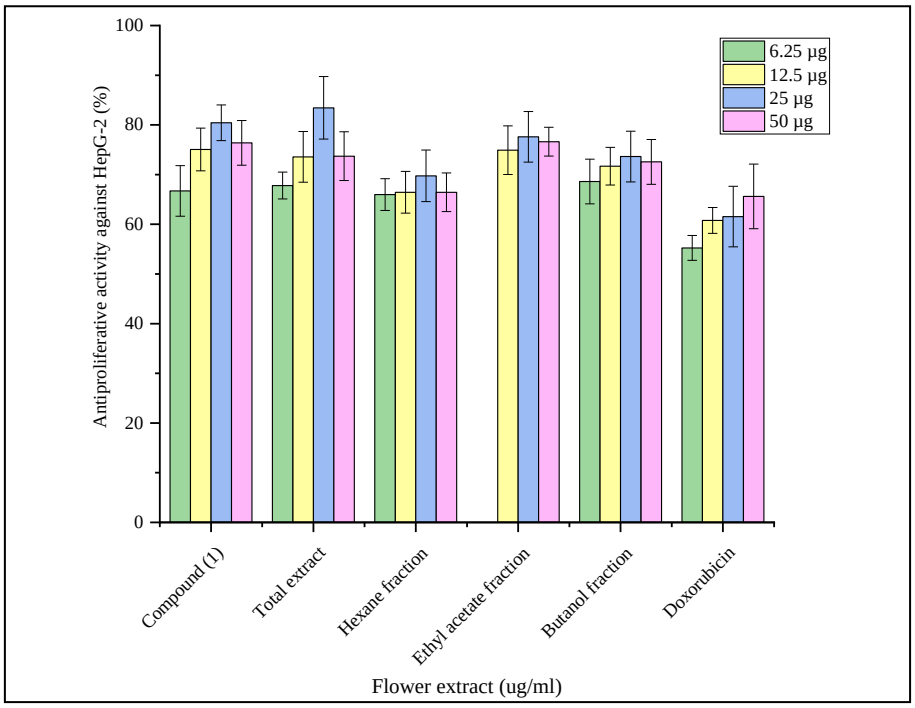

Figure 4. Dose dependent anti-proliferative activity of *C. dante purple* flowers compound (1), total methanolic extract and fractions against HepG-2 cancer cell-lines

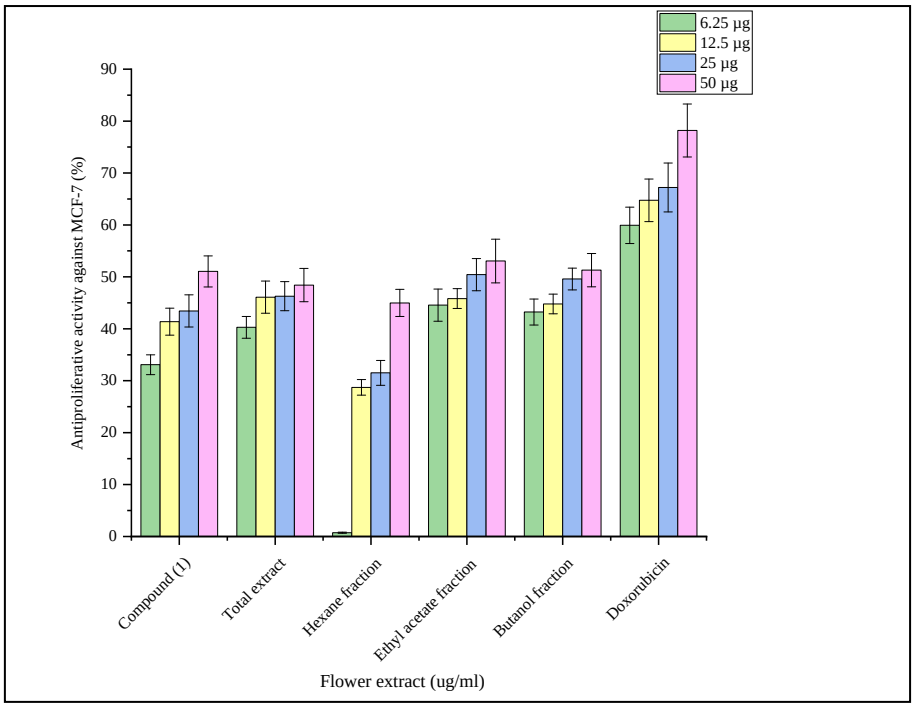

Figure 5: Dose dependent anti-proliferative activity of *C. dante purple* flowers compound (1), total methanolic extract and fractions against MCF-7 cancer cell-lines

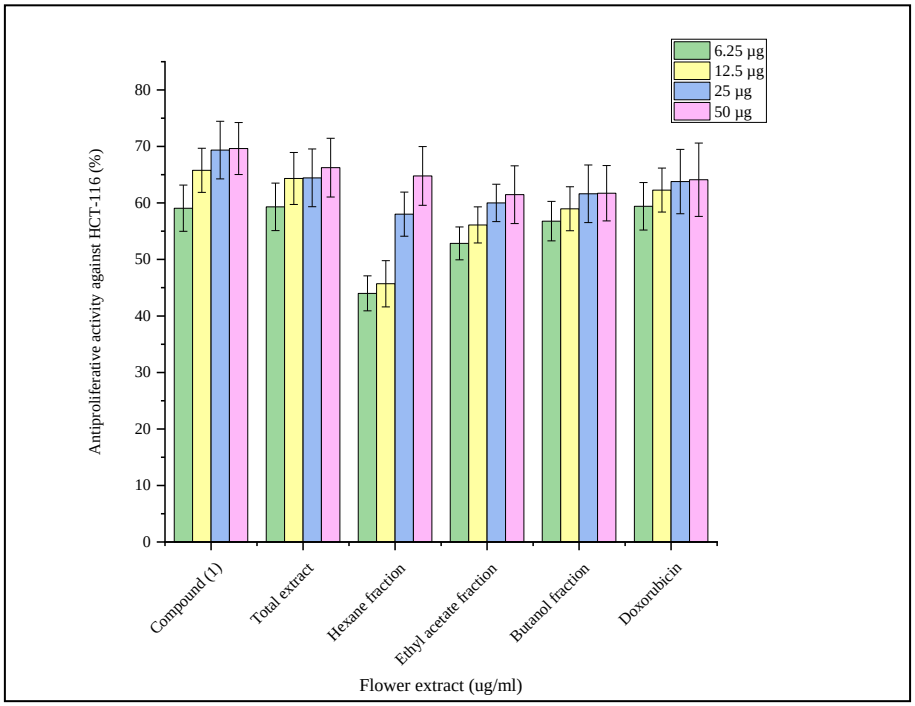

Figure 6. Dose dependent anti-proliferative activity of *C.dante purple* flowers compound (1), total methanolic extract and fractions against HCT-116 cancer cell-lines

Table 1: Anti-proliferative IC_{50} of *C. dante purple* flowers compound (1), total methanolic extract and fractions against HCT-116, HepG-2, and MCF-7 cancer cell-lines

Plant extract	IC_{50} (µl)		
	HepG-2	MCF-7	HCT-116
Compound (1)	4.68±0.31	7.57±0.6	5.29±0.62
Total methanolic extract	4.60±0.3	6.93±0.5	5.26±0.38
Hexane fraction	4.73±0.29	113.80±8.1	7.10±0.54
Ethyl acetate fraction	4.17±0.32	6.47±0.39	5.91±0.23
Butanol fraction	4.55±0.25	6.65±0.45	5.50±0.39
Doxorubicin	5.65±0.21	5.21±0.43	5.26±0.16

4. CONCLUSIONS

Six compounds were isolated from *C. dante purple* flowers for the first time. Analyses using MS and NMR were used to determine the chemical structures of the purified compounds. Antioxidant, anti-cholesterol, anti-inflammatory and anti-proliferative activities of the flowers compound (**1**), total methanolic extract, and fractions were evaluated for the first time. All the tested samples exerted potent DPPH free radical scavenging activity, anti-inflammatory activity and subsequently anti-proliferative activity against the tested cancer cell-lines with no cytotoxicity against the normal human skin cells. All tested samples exerted good cholesterol break down. This may be due to the fact that the majority of flavonoids and coumarins are normally present in *Chrysanthemum* flower extract and fractions. Overall results showed a noteworthy biological activity that should be exploited to find out a natural substitute for a lot of drugs, especially those with severe side effects.

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Conflicts of Interest: The authors report there are no competing interests to declare.

Author Contribution: Shaimaa Negm El-Dein: Methodology, Data visualization, Writing, Editing; **Amal H. Ahmed:** Supervision, Manuscript Editing; **Marwa S. Abu-Bakr**: Supervision, Manuscript Editing; **Asmaa Negm El-Dein**: Methodology,

Writing, Editing; **Hanem M. Awad**: Methodology, Writing; **Ehab A. Ragab**: Supervision, Manuscript writing and editing, Revision.

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