



## Chemical Investigation of Flavonoid, Phenolic Acids Composition and Antioxidant Activity of Mexican Marigold (*Tagetes erecta* L.) Flowers



Hanim A. Youssef\*, Safaa M. Ali, Mostafa I. Sanad, Dawood H. Dawood

Department of Agriculture Chemistry, Faculty of Agriculture, Mansoura University, Mansoura, Egypt.

**T**HIS study was geared towards investigating the chemical profile and *in vitro* antioxidant activity of methanol (MeOH) extract and its ethyl acetate (EtOAc) fraction of Mexican marigold (*Tagetes erecta* L.) orange flowers. A total of 29 known chemical compounds including phenolic and flavonoid compounds were identified and quantified using high performance liquid chromatography (HPLC) technique. The identification was carried out for EtOAc fraction for the first time. Pyrogallol and vanillic acid were highly abundant phenolic acids in flowers-methanolic extract and its EtOAc fraction amounted 37.57 and 3.34mg/g dry extract, respectively. On the other hand, apigenin-6-C-rhamnose-8-glucose and hesperidin were found to be the major flavonoid compounds detected in methanolic extract amounted 18.34 and 0.66mg/g dry extract, respectively. Furthermore, results asserted that the tested samples were more efficient in the three applied antioxidant methods compared with standard antioxidant references (Ascorbic acid and Butylated hydroxytoluene (BHT), especially EtOAc fraction which displayed the most activity against DPPH (2, 2-di phenyl-1-picrylhydrazyl) radical with  $IC_{50}$  value of  $32.5 \pm 0.12 \mu\text{g/mL}$ . These finding highlighted the use of *Tagetes erecta* flowers as a promising source of alternative bioactive compounds for both industrial and medicinal uses.

**Keywords:** *Tagetes erecta* (Marigold) flowers, Phenolics, Flavonoids, Antioxidant activity.

### Introduction

Oxidative damage caused by free radicals, play a fundamental role in the increasing of human disease. Free radicals share in tissue injury, proteins, lipids and DNA damage, which finally leads to catastrophic diseases like cancer [1]. Antioxidants can interfere with oxidative stress by inhibiting the lipid peroxidation, scavenging free radicals and chelating metals, subsequently prohibit the disease advancement [2]. The most widely used synthetic antioxidant compounds are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are suspected for being the responsible for many diseases like cancer and liver damage in lab animals [3]

Thus, there is a great need for natural antioxidant compounds with fewer side effects. Plants have been used for several years as a source of traditional medicine and as an excellent source for phytochemical compounds which contain functional antioxidant properties. Flower is an important plant part that had a great variety of natural antioxidant compounds like flavonoid and phenolic compounds [4]. Phenolics that have antioxidant activity can play a vital role in scavenging and neutralizing free radicals [5], and they are known to be mainly phenolic acids and flavonoids. These compounds have many biological activities such as antioxidant, anti-inflammatory, anticancer and antimicrobial. Flavonoids are secondary metabolites produced

\*Corresponding author e-mail: hanim 93 @mans.edu.eg.

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mainly by plants, include around 4500 identified compounds [6]. The beneficial value of flavonoids on health has been promoted for its antioxidant [1], anti-allergic, antitumor and anti-inflammatory activities [7]. *Tagetes erecta* (Marigold) is an ornamental plant belonging to the Asteraceae family, which widely distributed through the world and holds different colored flowers such as yellow, orange and brownish – yellow [8]. Many chemical compounds have been reported in genus *Tagetes* like flavonoid-O-glycosides [9]. Previous study carried for phytochemical screening in *Tagetes erecta* confirmed the presence of many pharmacological active compounds. It includes polyphenols, carotenoids, lutein, flavonoids and essential oils [8]. Additionally, many biological activities have been exhibited in genus *Tagetes* such as antimicrobial, anti-inflammatory and insecticidal activity [10].

The objectives of this study were to: (i) identify and quantify the phenolic compounds present in *Tagetes erecta* flowers, especially EtOAc fractions which haven't been cleared yet; (ii) evaluate and compare the antioxidant capacity by three common methods and (iii) determine the correlation relationship between antioxidant assays and total flavonoid and phenolic contents.

## Materials and Methods

### Chemicals and Reagents

#### Chemicals

- Aluminum chloride hexahydrate ( $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ), Ferric chloride ( $\text{FeCl}_3$ ), Ascorbic acid, Trichloroacetic acid ( $\text{CCl}_3\text{COOH}$ ), Potassium acetate ( $\text{CH}_3\text{COOK}$ ), Potassium ferricyanide ( $\text{K}_3[\text{Fe}(\text{CN})_6]$ ), Disodium ortho phosphate ( $\text{Na}_2\text{HPO}_4$ ), Monosodium orthophosphate ( $\text{NaH}_2\text{PO}_4$ ), Ammonium molybdate ( $(\text{NH}_4)_2\text{MoO}_4$ ), Methanol (MeOH), Ethyl acetate (EtOAc), Concentrated sulfuric acid ( $\text{Conc. H}_2\text{SO}_4$ ), Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) and Butylated hydroxytoluene (BHT) were purchased from El-Nasr Company for pharmaceutical chemicals.
- Gallic acid, Quercetin dihydrate and DPPH(2,2-di phenyl-1-picrylhydrazyl) (DPPH) radical were purchased from Sigma – Aldrich Company.

#### Reagents

- Folin-Ciocalteu's reagent was purchased from *Fluka*, Biochemika

### Plant Material

Orange flowering transplants of Mexican marigold (*Tagetes erecta* L.) were obtained from Green Valley Company for landscape and ornamental plants Dokki, Giza, Egypt in April 2016. Plant identification was carried out by Asso. Prof Mahmoud M. Kassem, department of Floriculture, Fac of Agriculture, Mansoura University, Mansoura, Egypt. These transplants were cultivated in the experimental farm, Fac of Agriculture, Mansoura University from April until September 2016. The flowers were cut in full flowering stage, separated, cleaned and airdried in the shade for two weeks. The air dried flowers were crushed into fine powder and stored in polyethylene pouches until usage.

### Plant Extraction

One kilogram of air dried powdered flowers was soaked in MeOH for 24h at room temperature. The obtained extract was filtered and the residue was re-extracted with MeOH three times at the same conditions. The extract was evaporated using rotary evaporator at  $45^\circ\text{C}$  to obtain crude methanolic extract. Fifty gm of crude MeOH extract was re-dissolved in MeOH and water (1:4) as a volume ratio and successively partitioned using EtOAc.

### Quantitative estimation of phytochemical compounds.

#### Total phenolic content

Total phenolic content was estimated spectrophotometrically using Folin – Ciocalteu reagent as mentioned before [11]. The reaction mixture contained 0.5 mL methanolic extract and its derived EtOAc fraction, 0.1 mL of Folin reagent and 0.5 mL of 7.5%  $\text{Na}_2\text{CO}_3$  solution. After incubation at room temperature in the dark for an hour the absorbance was measured at 740 nm. Each trial was performed in triplicates. The phenolic content was determined using external curve of gallic acid as a standard and results were expressed as mg gallic acid equivalent per gram dry weight (mgGAE/g DW).

#### Total flavonoid content

Aluminum chloride colorimetric method reported before [12] was used for total flavonoid content determination with some modification. Briefly, 2 mL MeOH, 0.3 mL of 10%  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  solution, 0.2 mL of 1M  $\text{CH}_3\text{COOK}$  and finally 2 mL of distilled water were added to 0.5 mL of flowers MeOH extract and its obtained EtOAc fraction. After incubation for 30 min at room temperature, the absorbance was measured

at 430 nm. Each trial was run in triplicates. Determination was calibrated with standard curve of prepared quercetin dihydrate solution and results were expressed as mg quercetin equivalent per gram dry weight (mg QE/g DW).

#### *Identification of phenolic and flavonoid compounds using HPLC technique.*

Phytochemical profile identification was performed in Food Tech Res Inst, Giza, Egypt. Flavonoid compounds were identified as reported before [13], while phenolic compounds were identified as reported earlier [14]. The identification of flavonoid and phenolic compounds in methanolic extract and its ethyl acetate fraction was performed using an Agilent Germany 1100 series HPLC equipped with a reverse phase C<sub>18</sub> column. HPLC method started with linear gradient with a flow rate 1 mL/min. The mobile phase systems were water/acetic acid (98:2, v/v) [A] and methanol/acetonitrile (50:50, v/v) [B]. The elution condition was 0-55 min, 5-70% [B]. The column temperature was 35°C and the injection volume was 20 µL. The Spectra of compounds were recorded at 330 nm to identify flavonoid compounds and 280 nm for phenolic compounds. All detected chromatograms were compared with external standards.

#### *Antioxidant activity*

##### *Reducing power assay*

The antioxidant activity of tested samples was measured through the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> ion according to [15] procedure. One mL of tested sample solution was mixed with 2.5 mL of sodium phosphate buffer (0.2mM, 6.6pH) and 2.5 mL of 1% K<sub>3</sub>[Fe (CN)<sub>6</sub>], then the mixture was incubated for 20 min at 50°C. Aliquots of 10% CCl<sub>3</sub>COOH (2.5mL) were added to stop the reaction. The reaction mixture 2.5 mL were added to 2.5 mL distilled water and 1 mL of 0.1% freshly prepared FeCl<sub>3</sub> solution was added and the reaction was completed after 10 min at room temperature. Absorbance was measured at 700 nm. Higher absorbance confirms higher reducing power. All measurements were run in triplicates. Ascorbic acid was used as a standard and EC<sub>50</sub> values of both the extracts and ascorbic acid were calculated.

##### *Total antioxidant capacity*

Total antioxidant capacity (ascorbic acid equivalent) was estimated as reported by [2]. Briefly, 0.3 mL of sample solution at a concentration of 500 µg/mL was mixed with one mL of the reaction mixture (28mM Na<sub>2</sub>HPO<sub>4</sub>,

4mM (NH<sub>4</sub>)<sub>2</sub>MoO<sub>4</sub> and 0.6mM H<sub>2</sub>SO<sub>4</sub>). Then, the tubes were kept in a hot water bath for 90 min. After cooling at room temperature the absorbance was measured at 765 nm. Each tested sample was performed in triplicates. Ascorbic acid standard curve was employed for calculation from the following equation: (y = 0.0036x - 0.1417, R<sup>2</sup> = 0.991).

#### *2,2 -di phenyl-1-picrylhydrazyl(DPPH) free radical scavenging method*

The hydrogen donating ability of tested samples was measured by decolorization of DPPH radical. This assay was performed according to method proposed by [16] with totally modification. Exactly, 60µM of freshly prepared DPPH methanolic solution was added to prepared samples with a ratio of (3DPPH: 1extract). After protecting from light at room temperature for 30min the absorbance was checked at 517 nm, the estimation was run in triplicates. A lower absorbance value of the reaction mixture indicates higher free radical scavenging activity. Antioxidant activity of tested samples was compared with those of standard Butylated hydroxytoluene (BHT).

Scavenging activity percentage value was calculated as follows:

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

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

#### *Statistical analysis*

Data were subjected to one way analysis of variance (ANOVA) and expressed as mean ± standard deviation at P<0.05 probability level. Pearson correlation test was used to illustrate the relationship between phytochemical compounds and antioxidant assays.

### **Results and Discussion**

#### *Quantitative estimation of phytochemical compounds*

It is well known that phenolic compounds share in antioxidant activity and therefore, it is quite important to determine the total phenolic content (TPC) and total flavonoid content (TFC) in tested samples.

In the present work, TPC and TFC were summarized in **(Table 1)**. As listed, EtOAc

fraction exhibited a remarkably TPC ( $125.18 \pm 0.19$  mgGAE/g DW) and TFC ( $112.81 \pm 1.74$  mgQE/g) compared with its MeOH extract. Flowers MeOH extract contained higher TFC than TPC, however flavonoid compounds are a subclass of phenolics. That is can be illustrated by compounds rearrangement which occurring during the flowering metabolism leading to a decrease or change in the phenolic compounds structures [17]. The comparison with literature showed that the obtained results were in accordance with those found in [18, 19]. They found that TPC and TFC in alcoholic extracts of marigold flowers were  $62.33 \pm 1.81$  mg GAE/g and  $68.9$  mgRE/g, respectively.

On the other hand, the results in (Table 1) were completely differ from those found in the work of Hemali and Sumitra [20], they stated that TPC and TFC values in marigold flowers EtOAc extract were  $50$  mgGAE/g and  $202.68$  mg/g, respectively. Another study undertaken by Kaewseejan and Siriamornpun [21] on one of Asteraceae family members documented the superiority of EtOAc fraction in TPC and TFC, which support our results.

The difference noticed in TPC and TFC in the present work and previous studies found in literature might be due to the type of phenolic compounds and their solubility in different solvents with different polarities[22].

#### Identification of phenolic and flavonoid compounds using HPLC technique.

The identified compounds in marigold (*Tagetes erecta* L) flowers were depicted in (Table 2), HPLC analysis applied in this study achieved the identification of 14 flavonoid compounds and 15 phenolic compounds.

Fourteen flavonoid compounds were identified. Flavones involved the highest number of identified flavonoid compounds. The major flavonoid compound was apigenin-6-C-rhamnose-8-glucose (flavone) followed by hesperidin (flavanone) amounted  $18.34$  and  $2,94$  mg/g dry extract, respectively found in MeOH extract. Contrary, EtOAc fraction contained low amounts of detected flavonoid compounds compared with its methanolic extract and the lowest one was naringenin (flavnone) amounted  $0.0013$  mg/g dry extract.

Fifteen phenolic acids involving hydroxybenzoic (HBA) and hydroxycinnamic acids (HCA) were also identified. Among the identified phenolic acids, the most predominant phenolic acids in amount were pyrogallol and gallic acid (HBA) detected in flower methanolic extract. Their amounts were  $37.57$  and  $9.9$  mg/g dry extract, respectively. In addition, ellagic acid and isoferulic acid weren't found in the same extract. The most widely occurring phenolic acid in EtOAc fraction was vanillic acid (HBA) amounted  $3.34$  mg/g dry extract. Alpha- coumaric (HCA) was the lowest detected phenolic acid in amount  $0.014$  mg/g dry extract found in EtOAc fraction.

The existing of caffeic acid, *p*-hydroxy benzoic acid, gallic acid, ferulic acid, quercetin and rutin and absence of vanillic acid and apigeninin marigold flowers was noticed [19]. Additionally, rutin, *p*-coumaric acid, caffeic acid, ferulic acid and vanillic acid have been found in HPLC results of wild *Tagetes minuta* 80% methanolic extract [23].

To the best of our knowledge, this is the first report cleared the phytochemical profile of

**TABLE 1. Total phenolic and flavonoid contents in marigold flowers.**

Samples	Phytochemical compounds	
	TPC mgGAE/g DW	TFC mgQE/g DW
Marigold flowers MeOH extract	$57.52 \pm 1.42$	$67.88 \pm 2$
Marigold flowers EtOAc fraction	$125.18 \pm 0.19$	$112.81 \pm 1.74$

Values represented the means  $\pm$  standard deviation (n=3).

**TABLE 2. Identification of phenolic and flavonoid compounds using HPLC technique in marigold flowers.**

Compounds	MeOH extract	EtOAc fraction	R <sub>t</sub>
<b>Flavones</b>			
Apig-6-rhamnose-8-glucose	18.34	0.057	12.06
Apig-6-arbinose-8-galactose	0.31	0.046	11.64
Apig-7-neohespiroside	1.52	0.025	13.143
Apigenin	0.034	0.0046	16.582
Acacetin neo-rutinoside	0.652	0.072	15.097
<b>Flavanones</b>			
Naringin	1.47	0.22	12.327
Naringenin	0.56	0.0013	15.014
Hesperidin	2.94	0.66	12.473
Hesperetin	1.44	0.016	15.313
<b>Flavonols</b>			
Rutin	0.39	0.044	12.657
Quercetin	0.19	0.021	14.875
Quercitrin	0.447	0.041	13.446
Kaempferol	0.031	0.013	16.193
<b>Flavanol</b>			
Catechin	0.995	0.68	8.686
<b>Hydroxy benzoic acids</b>			
Pyrogallol	37.57	1.06	6.995
Gallic acid	9.9	0.43	7.078
Protocatechuic acid	4.12	0.45	8.395
<i>P</i> -OH-benzoic	1.3	0.769	9.799
Vanillic	0.789	3.34	10.167
Ellagic	nd	0.71	13.436
Salicylic	0.05	0.76	16.453
<b>Hydroxy cinnamic acids</b>			
Coumarin	0.18	0.046	14.504
3,4,5tri methoxy cinnamic	3.2	0.213	14.208
Alpha-coumaric	0.272	0.014	13.279
Isoferulic	nd	0.14	12.167
Ferulic	0.33	0.098	11.787
<i>P</i> -coumaric	0.31	0.277	11.596
Caffeic	4.95	0.47	10.344
Chlorogenic	5.58	0.19	10.1

\*Expressed as mg/g extract, nd: not detected.

marigold flowers EtOAc fraction.

The difference in the amounts of bioactive phytochemical molecules and occurrence or absence of any bioactive phytochemical molecule could be obviously attributed to some agents such as plant part, environmental conditions, maturity at harvest, extraction method and used solvent [24].

#### *Antioxidant activity*

The antioxidant activity of samples under investigation was evaluated by three complementary different methods to take into consideration the various mechanisms of antioxidant action.

#### *Reducing power assay*

The antioxidant activity of investigated samples was evaluated using reducing power assay. Reductants, which have reduction ability, react with potassium ferricyanide ( $\text{Fe}^{3+}$ ) to form potassium ferrioxalate ( $\text{Fe}^{2+}$ ), which then reacts with ferric chloride to form ferrous complex that has a maximum absorption at 700 nm. As detailed in (Table 3), it could be seen that all samples showed significant reduction ability compared

with standard antioxidant compound ascorbic acid. These results demonstrated that the tested samples had a remarkable reduction ability and can be used for neutralizing free radicals. Therefore, it is confirmed that the antioxidant properties of any tested sample is directly correlated with their reduction ability [25]. Flowers EtOAc fraction exhibited the highest reduction ability with the lowest calculated  $\text{EC}_{50}$  value  $146.53 \pm 8.54 \mu\text{g/mL}$ . Contrary to the results of [26] they reported that the reducing power of marigold flower ethanolic extract was higher than its EtOAc fraction

#### *Total antioxidant capacity*

This assay measures the reduction capacity of  $\text{Mo(VI)}$  to  $\text{Mo(V)}$  by antioxidant compounds. The results were calculated on the basis of ascorbic acid as a standard antioxidant compound, and were expressed as mg AAE/g dry extract. The highest reduction capacity as seen in (Table 3) was detected in flowers EtOAc fraction  $396.31 \pm 2.84$ , which is nearly 1.3 fold higher than those detected in its methanolic extract. The donated results in Table 3 showed opposite tendency to those detailed by [27, 2], they found

**TABLE 3. *In vitro* antioxidant activity evaluation of marigold flowers.**

Samples	RP <sup>A</sup>	TAC <sup>B</sup>	DPPH <sup>C</sup>
Marigold flowers MeOH extract	158.13±6.58	298.35±2.63	113.32±7.89
Marigold flowers EtOAc fraction	146.53±8.55	396.31±2.84	32.5±0.12
BHT	-	-	144.74±8.78
Ascorbic acid	191.78±1.67	-	-

A means reducing power assay and expressed as effective concentration at which the absorbance is 0.5 ( $\text{EC}_{50}$   $\mu\text{g/mL}$ ).

B means total antioxidant capacity and expressed as mg ascorbic acid equivalent per g extract (mgAAE/g).

C expressed as half maximal inhibitory concentration ( $\text{IC}_{50}$   $\mu\text{g/mL}$ ).

Values represented the means  $\pm$  standard deviation (n=3).

**TABLE 4. Correlation coefficients (r) between phytochemical compounds and antioxidant assays in marigold flowers.**

	Phytochemicals		Antioxidant assays		
	TPC	TFC	DPPH	TAC	RP
TPC	1	-	-	-	-
TFC	***0.999	1	-	-	-
DPPH	***-0.996	***-0.995	1	-	-
TAC	***0.998	***0.995	***-0.992	1	-
RP	-0.675	-0.691	0.632	-0.665	1

\*\*\*p<0.001, extremely significant correlation.

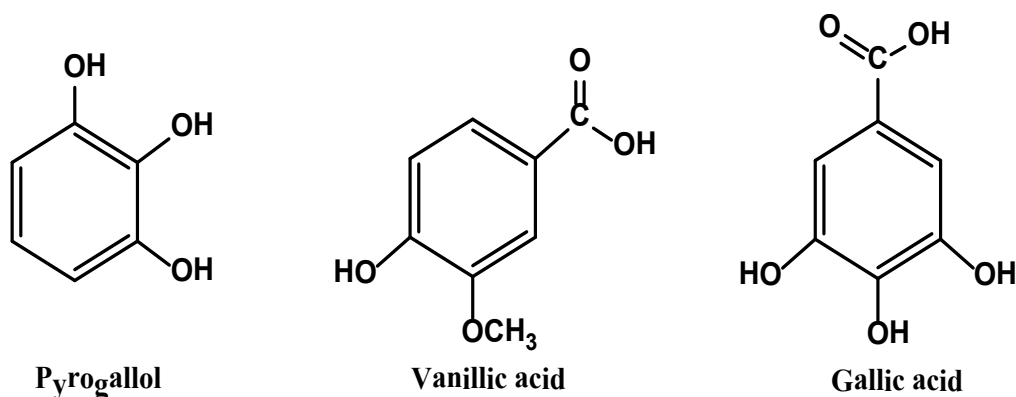


Fig.1. Structure of the highest concentration of phenolic acids.

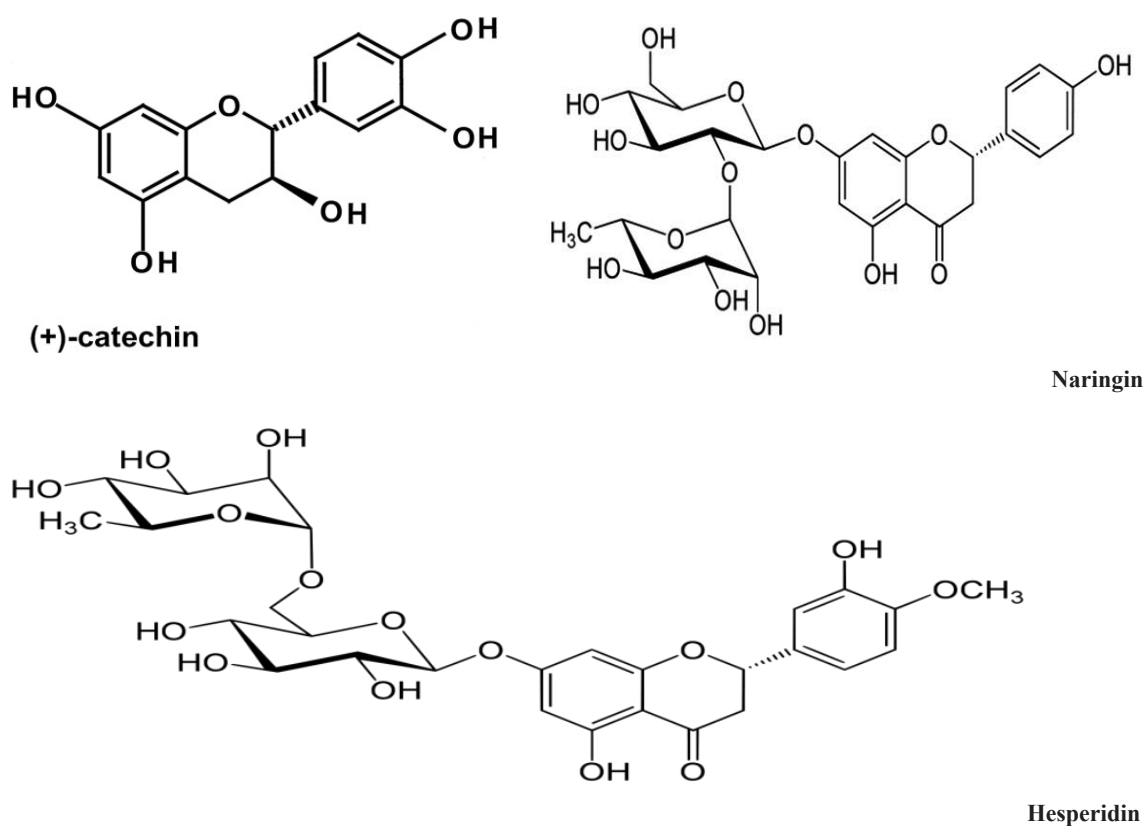


Fig. 2. Structure of the highest concentration of flavonoid compounds.

that the total antioxidant capacity in marigold flowers methanolic extract was  $183.62 \pm 0.001$  and  $493 \text{ mg AAE/g dry extract}$ , respectively.

As far as our literature survey we could ascertain that there weren't any previous data documented total antioxidant capacity in marigold flowers EtOAc fraction for discussion.

*2,2-di phenyl-1-picrylhydrazyl (DPPH) free radical scavenging method*

The DPPH radical is a stable lipophilic free

radical has been widely used to measure the non-enzymatic antiradical activity of plant extracts [24]. Results showed that the investigated samples were more potent to scavenge DPPH radical than standard antioxidant reference BHT. Samples competence in DPPH reduction was ordered as follows: flowers EtOAc fraction, flowers methanolic extract, BHT with  $IC_{50}$  values  $32.5 \pm 0.12$ ,  $113.32 \pm 7.89$  and  $144.74 \pm 8.78 \mu\text{g/mL}$ , respectively as summarized in (Table 3).

The gathered results in **Table 3** were very close to those found in the publication of [27,20]. They reported that the  $IC_{50}$  values of marigold flowers methanolic and EtOAc extracts against DPPH radical were 117.47 and 30 $\mu$ g/mL, respectively.

The antioxidant activity of tested samples appeared similar trend with both TPC and TFC, confirming that phenolic and flavonoid compounds are motivation compounds for antioxidant activity. The excellence of EtOAc fraction in applied antioxidant methods could be explained by the solubility of antioxidant molecules that is dependent on solvent polarity. According to antioxidant methods we demonstrated that phenolic compounds with strong antioxidant properties were more soluble in medium polar solvent EtOAc.

The antioxidant properties of phenolic compounds are mainly refer to their redox properties, which give them the ability to work as reducing agents, singlet oxygen quenchers and hydrogen donors[28]. Antioxidants work mainly through two mechanisms: single electron transfer and hydrogen atom transfer. Reducing power and DPPH assays employ the single electron transfer mechanism and widely used to evaluate the potential antioxidant activity of plant extracts [29]. Moreover, electrons or hydrogen atoms transfer from antioxidant molecules based on the number of phenol groups, position and number of hydroxyl groups [30].

In general, it is supposed that the antioxidant activity of flavonoid compounds are mainly due to the presence of 2, 3 double bond in integration with 4-keto group and 3-OH group in the ring C and the additional OH groups at 3',4' positions on ring Band at 7 position in the ring A [31]. Thus, the low amounts of flavanones, rutin and quercetrin detected in flowers EtOAc fraction compared with its methanolic extract might be the reason for flowers EtOAc fraction superiority in all applied antioxidant methods. Consequently, phytochemical screening for bioactive molecules in plant extracts is one of the needed techniques used to explore the antioxidant compounds in plant extracts.

Several antioxidant assays have been developed to determine the potential antioxidant activity of plant extracts. However, each method provides an estimation of antioxidant activity that is dependent upon the reaction time, the complexity of the reaction mixture and the

used method. Thus, a single antioxidant method can't clear the antioxidant properties of plant extracts[19].

#### *Correlation analysis between phytochemical compounds and antioxidant assays.*

Antioxidant effectiveness of marigold is associated with the quantity of phenolic and flavonoid compounds as these compounds act as free radical scavengers during the oxidation reaction. DPPH radical scavenging activity, total antioxidant capacity, and reducing power assay all have been used to evaluate the antioxidant activity of marigold flowers. Therefore, to make further understanding of the relationship between antioxidant activity, TPC and TFC correlation analysis between these phytochemicals and performed antioxidant assays have been done.

The analysis of experimental data cleared an extremely significant correlation ( $p < 0.001$ ) between TPC, TFC, DPPH and total antioxidant capacity assays. These results reflect the liberality of phenolic and flavonoid compounds in marigold antioxidant activity. It is interesting to narrate that in current finding a non-significant negative correlation ( $p < 0.05$ ) was found between TPC, TFC and reducing power assay. The deviation of observation from usually studied trend that TPC and TFC are directly linked to antioxidant activity, this is might be due to the fact that other bioactive compounds of plants (which are not polyphenol in chemical nature) might be reason for reducing power in marigold flowers.

Some publications documented the relationship between TFC, TPC and antioxidant activity in plant extracts [19] whereas, others found no relationship [32]. The TPC doesn't represent all antioxidant components in the extract, which illustrate the unclear relationship between phenolic compounds and different antioxidant assays [33]. The antioxidant activity of plant extracts depends on antioxidant compounds content in the extract, the structure and the reaction between these compounds and the used chemicals in the antioxidant assay [34].

These results were in agreement with previous study by [5] who reported that there were no relationship between TPC and reducing power ability of *Allium sativum* and *Zingiber officinale*. Similar to our finding, another study undertaken by [19], they reported that the correlation coefficients ( $r$ ) between TFC, TPC and DPPH assay in defatted *Tagetes erecta* L. (marigold)



flowers were 0.982, 0.901, respectively.

Differences in correlation coefficients between TPC and applied antioxidant methods could be elucidated by different responses of phenolic compounds to different antioxidant assays as well as the nature of produced products by the reaction system [36].

### Conclusion

*Tagetes erecta* L. flowers belonging to family Asteraceae are enhanced in phenolic compounds that are known for their healthy benefits. This is the first detailed report on phenolic compounds from *Tagetes erecta* flowers EtOAc fraction. Twenty nine compounds were identified and quantified. The exploration of biological activities cleared that *Tagetes erecta* flowers had adorable antioxidant activity especially EtOAc fraction. Overall, results showed that investigated samples can serve as a valuable natural source of bioactive phenolic compounds and antioxidant activity with potential application in food and pharmaceutical industry

### Conflicts of interest

All authors disclose any potential sources of conflict of interest.

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## التحقق الكيميائي للمركبات الفلافونيدية، و الأحماض الفينولية والقدرة المضادة للأكسدة لأزهار نبات القطيفة

هاتم عبدالحليم عبدالعاطي يوسف، صفاء محمد علي، مصطفى ابراهيم سند و داود حسني داود  
قسم الكيمياء الزراعية - كلية الزراعة- جامعة المنصورة – المنصورة – مصر.

تهدف الدراسة الحالية إلي التعرف علي المركبات الفعالة الموجودة في أزهار نبات القطيفة باستخدام التحليل الكروماتوجرافي عالي الأداء HPLC.

أوضحت نتائج التحليل الكروماتوجرافي للمستخلص الميثانولي ومستخلص خلات الإيثايل المنفرد منه وجود 29 مركب وتضمنت هذه المركبات أحماض فينولية ومركبات فلافونيدية.

وقد اشارت نتائج التحليل الكروماتوجرافي إلي احتواء المستخلص الميثانولي علي أعلى نسبة من الأحماض الفينولية التالية: Pyrogallol, Gallic acid and Chlorogenic acid بينما احتوي مستخلص خلات الإيثايل المنفرد منه علي أعلى نسبة من Vanillic acid.

تميز المستخلص الميثانولي باحتوائه علي نسبة عالية من المركب الفلافونيدي 8-rhamnose-6-apig-glucose بينما ال hesperidin كان المركب السائد في مستخلص خلات الإيثايل المنفرد منه

تم تقييم القدرة المضادة للأكسدة معمليا باستخدام ثلاث طرق مختلفة وتميز كل من المستخلص الميثانولي ومستخلص خلات الإيثايل المنفرد منه بفعالية عالية كمضادات أكسدة طبيعية مقارنة بمضادات الأكسدة الصناعية (Ascorbic acid and BHT) المستخدمة في عملية التقييم.