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# Assessment of Phytochemical Content and Antioxidant Activity of the Extracts Collected from the Leaves and Flowers of *Hypericum scabrum*

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## ABSTRACT

To study the bioactive substances of the *H. scabrum*, ethanolic and aqueous extracts of leaves and flowers were analyzed for their phytochemical contents and antioxidant actings. The antioxidant potiential was estimated using four methods, including DPPH radical, ABTS decolorization assay, reducing power and total antioxidant capacity. The results exhibited that the ethanolic flower contain higher amounts of total flavonoids (4.675 mg. $g^{-1}$) and saponin (29.572 mg.$g^{-1}$) contents, whereas, the water flower extract hold less amount of total flavonoids compounds (1.223 mg. $g^{-1}$). Similarly, our results exposed that the antioxidant activities detected by DPPH and ABTS methods of the ethanolic extract of flower, were evidently better than the rest of extracts. Significant high relationship were noticed between total phenolic amount and total antioxidant activity, and between total flavonoid content and DPPH inhibition assay. Principal component analysis reveals 91.49 of total variance which indicates that the different extracts were well distinguished by their phytochemical substance contents and antioxidant effects.





## INTRODUCTION

The data of World Health Organization (WHO) displays that 80% of world people depends on folk medication using medicinal plants and over that, most of the sources of anticarcinogenic, antimicrobial and antiflammation drugs are consequent from plants (Shafaghat, 2011). The genus Hypericum L. is a member of the Hypericaceae family, as well as holding 484 species of annuals, perennials, shrubs andinfrequently trees, widespread in warm-temperate areas throughout the world (Crockett and Robson, 2011). *Hypericum* species are long regarded as healing herbs (Bayramoglu *et al.*, 2014). Many species of this genus were utilized in disease dealing in several nations and have a very important place among helpful meditative herbs. The full plant extract has antidepressive impacts on neurotransmitter levels in the brain(Çakir *et al.*, 1997; Ganji *et al.*, 2017).

*Hypericum scabrum* L. is a perennial plant which is spread in dry rocky slopes and open woodland. Recent studies highlight the medicinal potential of this species, mainly due to its cytotoxic activity and anti-inflammatory, antibacterial, antifungal, antitumor, antioxidant, and antidepressant feateurs.Previous phytochemical works on *Hypericum scabrum* performed the separation and documentation of several classes of phytochemicalswith pharmaceutical importance, comprisingessential oil, fixed oil,phenolic and flavonoid compounds (Davis *et al.*, 2000; Kizil *et al.*, 2004; Ayan *et al.*, 2009; Eslami *et al.*, 2011; Shafaghat, 2011; Jiang *et al.*, 2015; Tanaka *et al.*, 2004; Çakir *et al.*, 1997; Shafaghat, 2011; Ghasemi Pirbalouti *et al.*, 2014; Hamzeloo-Moghadam *et al.*, 2015).

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The quality and amount of phytochemical in plants aregreately influenced by environmental, genetic makeup, physiological elementsand infection bypests. The bioactive elements from *Hypericum* sp. are produced and/ or stored in diverse secretory constructions, including translucent glands, dark glands and secretory canals located in different organs of the plants (Murch and Saxena, 2006; Liu *et al.*, 2015; Bayramoglu *et al.*, 2014). Secondary metabolites are regularlyformed in plant cells as a reaction to defend plants from different environmental stresses causedby biotic (Bacteria, Fungi and insects) and abiotic ( temperature,moisturee, UV light, and heavy metal)(Liu *et al.*, 2015). Herbal extracts are a good source of antioxidant compounds. A research showed the antioxidantcapacity of ethanol extracts of *H. scabrum* L. against the DNA and protein oxidation (Kızıl *et al.*, 2011). To the best of our knowledge, however, there is no report on the phytochemical contents of the ehanolic extract from the flower and leaf of *H. scabrum* from Iraq and on their antioxidant activities.The plant of the current investigation is to determine the accumulation of the phytochemical contents in leaf and flower *H. scabrum* and their antioxidant activities.

## MATERIALS AND METHODS

### Plant materials

*H. scabrum* were gathered from rocky regions within the Basne district of Sharbazher province, Sulaimani, Iraq, in august, 2018. The species were identified by Asssistant Prof. Dr. Rupak Tofiq, Sulaimani University, College of Agricultural Sciences, Department of Horticulture. The sample was recorded by a voucher number (KA-1) and stored at the herbarium of the college of Agricultural Sciences. The specimens were dried in the shade at 24º C for 7 days, and they were grounded (Tahir *et al.,* 2017). The extracts were collected by soaking in solvents including distilled water and pure ethanol. For extracting, 1 g of *H. scabrum* powder is transferred to the glass test tube, and 15 mL of distilled water and pure ethanol are added to the powder and the lid is closed with parafilm. Glass tubes remained on the shaker for 2 hours, then incubated at 6°C for 16 h. After incubation, the samples were shaked for two other hours. The mixtures centrifuged at 10°C for 10 min and the supernatant was filtered. The solvent was removed by rotary evaporator at 40 °C.The dried samples were weighted (Tahir *et al.,* 2018).

### Measurement of total phenolic compounds

The Folin-Ciocalteau method is utilized to determine the concentration of total phenolic acids compounds (Singleton *et al.*, 1999). The 0.11 mL of extract was mixed with 1.6 ml Folin-Ciocalteau (0.2 N). After 7 minutes of incubation, 1.3 mL of sodium carbonate solution (10%) was supplemented and the solution was stored in the dark at 40°C for 45 min. The absorptions of samples were determined with a spectrophotometer at 765 nm. Various concentrations of gallic acid were prepared and employed as a standard and its absorption determined at 750nm.The quantity of phenolic compounds in samples was determined by calibration curve andexpressed in µg gallic acid equivalent/ g dry sample.

### Determination of total flavonoid compounds:

The $AlCl_3$ method was used for determination of the total amount of flavonoid of the aqueous and ethanolic leaf and flower extracts by using three triplicates (Madaan *et al.*, 2011). Three ml of a solution composing of 0.9 ml of methanol 80%, 0.3 ml of 2% $AlCl_3$, 0.07 ml of 1 M potassium acetate and 1.73 ml of autoclaved distilled water was supplemented to 30 µl of the sample extract. The mixture was strongly shaken and the mixture was incubated at room temperature for 30 min. The absorbance of the reaction combination was estimated at 415 nm. Different concentrations of quercetin were applied to produce the reference curve. The regression equation of linear regression of standard curve of quercetin, was created by MS Excel software and applied to calculate the total flavonoid content in all samples. Total flavonoid amounts were expressed as quercetin equivalents in mg per gram dry sample.

**Calculation of total tannin content**

The condensed tannin contents were established by method of Broadhurst and Jones (1978) with some modifications. A volume of 40µL of extract was supplemented to 2 mL of a solution of vanillin (4% in methanol) and 1 mL of concentrated HCl. After 19 min of standing, the absorbance was achieved at 500 nm. The condensed tannin amount was determined using the reference curve of catechin compound and it expressed as equivalents in mg per gram dry sample.

**Determination of total saponin contents**

This bioactive compounds were computed by vanillin method using saponin as reference component (Du *et al.*, 2018). A volume of 0.75 mL of a 4% vanillin solution was added to 35 µL of extract and put it on ice, followed by the addition of 4 ml of 72% (w/v) sulfuric acid. The mix was allowed to stand for 10 min, at 60 C and then cooled in ice-cold water for 5 min. After incubation, the mixture absorbent was read at 540 nm. The total saponin contents was calculated using the standard curve of saponin compound and it is expressed as equivalents in mg per gram dry sample.

**Determination of antioxidant activity**

**Measurement of radical scavenging by DPPH:**

The antioxidant ability of different extracts was determined using the method DPPH radical-scavenging as defined by Shimada *et al.*(1992). Two ml of DPPH solution ($6x10^{-5}$ M) was mixed with 10 µl of sample. A control containing methanol and DPPH solution was also realized. Different concentrations of ascorbic acid (2.0-12.0 $\mu g.mL^{-1}$) were prepared and used as a reference. All solutions incubated for 30 min at 23°C and then Absorbances were done at 517 nm. The inhibition percentage of samples was computed from the data of absorbance bythe equation:

% inhibition = [(Abs control-Abs sample)/Abs control] × 100

**Measurement of radical scavenging by ABTS radical cation**

The free radical scavenging activity of leaf and flower samples was determined by ABTS radical cation decolorization assay(Re *et al.*, 1999). Various concentrations of samples and reference were prepared. ABTS radical cation was made by the mixing of ABTS (7mM) in water and potassium persulfate (1:1). The ABTS radical cation solution was reserved in the dark at 21°C for 15h before use. For obtaining an absorbance of 0.700±0.002 at 734 nm, the ABTS radical cation solution was diluted with methanol in ratio 1:41. After the addition of 7 µl of organextract to 2.993 ml of diluted ABTS radical cation solution, the absorbance was read at 8 min after the initial mixing, at 734 nm. A control solution contained 7 µl of methanol and 2.993 ml of diluted ABTS radical cation solution. Methanol was used as a blank. All tests (control and sample) were repeated three times. Trolox was applied as a standard substance. Percent of inhibition of absorbance at 734 nm was calculated using theformula,

ABTS cationscavenging (%) = [(Abs control–Abs extract)/Abs control]×100

**Estimation of reducing power**

Various concentrations of the aqueous and ethanolic leaf and flower extraction (10mg/ml) were prepared and the 100 µl of the extract were mixed with 1.4 ml of phosphate buffer (200 mM, pH 6.6) and 1.4 ml of potassium ferricyanide (1%). This solution was kept at 50ºC in water bath for 22 minutes. After cooling, 1.4 ml of trichloro acetic acid (10%) was supplemented and centrifuged at 4000 rpm for 9 min whenever necessary. A volume of 1.5 ml of supernatant layer was mixed with 1.5 ml of distilled water and 0.4 m of a freshly prepared ferric chloride solution. The absorbance was determined at 700 nm. Blank was set in a similar manner without extract. Ascorbic acid in various concentrations was utilized as a reference (Oyaizu, 1986).

**Assessment of total antioxidant activity via phosphomolybdenum method**

The total antioxidant capacity of the leaf and flower extracts was assessed according to the method described by Prieto *et al.* (1999). A volume of 0.1 mL of sample solution was combined with 3.9 mL of a mix solution (0.6 M sulfuric acid, 0.028M sodium phosphate, and 4 mM ammonium molybdate). In case of blank, 0.1 mL of distilled water or ethanol was used as a substitute for plant extract. The tubes were located at 95°C for 85 min. After this period, the samples were cooled, and the absorbance was read at 695 nm against blank, in UV- spectrophotometer (Shimadzu, Japan). Ascorbic acid in various concentrations was utilized as a reference. The curve of reference was used to determine the amount of total antioxidant ability and it expressed as equivalents in mg per gram dry sample.

## RESULTS AND DISCUSSION

**Phytochemical contents**

The results of the total phytochemical contents of the examined organs extract are stated in Table 1. The amount of total phenols, flavonoids, tannins and saponins in various extracts, expressed as milligram gallic acid, quercetin, catechin, and saponin equivalents per gram of dry extract. Total phenolic, flavonoid, tannin and saponin contents in vitro were determined for ethanol and water extract of leaf and flower *Hypericum scabrum*. Significant differences ($P< 0.05$) were detected between the different extracts for phytochemical contents (Table 1). The values of phytochemical compounds were ranged from 3.066 to 5.553, 1.223 to 4.675, 4.912 to 29.572 and 0.715 to 1.904 mg. $g^{-1}$ for total phenolic, flavonoid, tannin and saponin contents, respectively. The results designated that ethanolic flower extract of *H. scabrum* has the highest total flavonoid and saponin contents when compared to the other *H. scabrum* extracts. While, water flower and leaf extracts showed the lowest total flavonoid and saponin contents. The output data demonstrated that flower extract contained higher amount of phytochemical contents than leaves. In addition, the plant *H. scabrum* have high level of saponin and low levels of tanins. These results indicated the influence of the extraction solvent on the total content of phytochemical compounds extracted. Heshmati *et al.* (2018) described a flavonoid amount of 30.8 mg quercetin equivalent/g of water extract. This quantity is superior than the value initiate in the current study (2.39 $mg.g^{-1}$). On the other hand, the content of phenols constituents in our research was greater than the amount of phenols components in the study of Heshmati *et al.* (2018).

**Antioxidant activities**

Different solvents, ranged from higher polarity to lower polarity, including water and ethanol, extracts was applied for the study of antioxidant effeciency.

**DPPH scavenging activity**

The effect of an antioxidants on DPPH radical scavenging is contributed to their donating of hydrogen DPPH substance. When the 2,2-diphenyl-1-pycrylhydrazyl (DPPH) is mixed with the extract and then the extract reduces the DPPH substance with the change of its violet color. Free radical scavenging abilities of the studied extracts was determned by DPPH method and the results are revealed in Figure 1. As stated by the results obtained from leaf and flower of *H. scabrum*, significant variations ($P< 0.05$) were detected between the antioxidant activity of diverse extracts. The percent of inhibition in ethanolic extract was higher than that obtained by water extraction. Ethanolic extract of *H. scabrum* flower possessed the strongest antioxidant activities compared to water extract. Similar finding was observed by Heshmati *et al.* (2018).

The powerful antioxidant properties of ethanolic materials extracts are believed to be a result of phenolic compounds including flavonoid, flavonol, phenolic acid, etc.. In general, fractions with greater radical scavenging displayed a higher phenolic amount (Salvador *et al.*, 2001).

**Table 1. Phytochemical contents in leaf and flower extracts of *H. scabrum*.**

| Samples | TPC ($mg.g^{-1}$) | TFC ($mg.g^{-1}$) | TTC ($mg.g^{-1}$) | TSC ($mg.g^{-1}$) |
|---|---|---|---|---|
| Ethanolic extract-Flower | 4.913 b | 4.675 a | 0.899 b | 29.572 a |
| Aqueous extract-Flower | 5.553 a | 1.223 d | 1.904 a | 6.254 c |
| Ethanolic extract-Leaf | 3.066 c | 3.846 b | 0.715 b | 9.929 b |
| Aqueous extract-Leaf | 4.896 b | 2.391 c | 0.989 b | 4.912 d |
| Pr > F | 0.001 | 0.000 | 0.001 | 0.000 |
| Significant | Yes | Yes | Yes | Yes |

Averages with different letters in a column are statistically significant at level P<0.05.

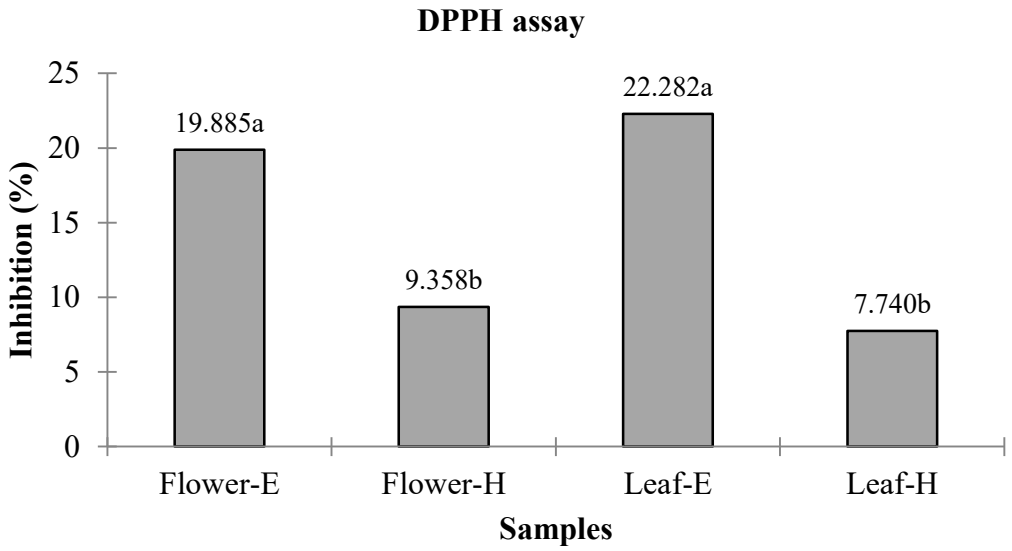


**Figure 1. Antioxidant activity of leaf and flower of *H. scabrum* extracts using DPPH method. E: Ethanolic extract and H: Aqueous extract**

**Measurement of free radical-scavenging ability by ABTS method**

The result of the antioxidant efficiency of the plant extracts diverges depending on the nature of the solvent and methods of analysis. The outcomes obtained from the ABTS assay are exposed in Figure 2**.** As seen in Figure 2, high significant difference among the extracts was revealed for the percentage of inhibition. The inhibition percentage extended from 43.039 to 89.122%. The highest inhibition was identified in the ethanolic extract collected from the flowers and the minimum in aqueous extract acquired from the leaves. The antioxidant activity of the flower extract measured by ABTS method was significantly higher than the leaf extract. This result can be attributed to the higher phenolic, flavonoid, and saponin contents of the flower. Moreover, ethanolic extracts exhibited greater antioxidant performance than water extracts.

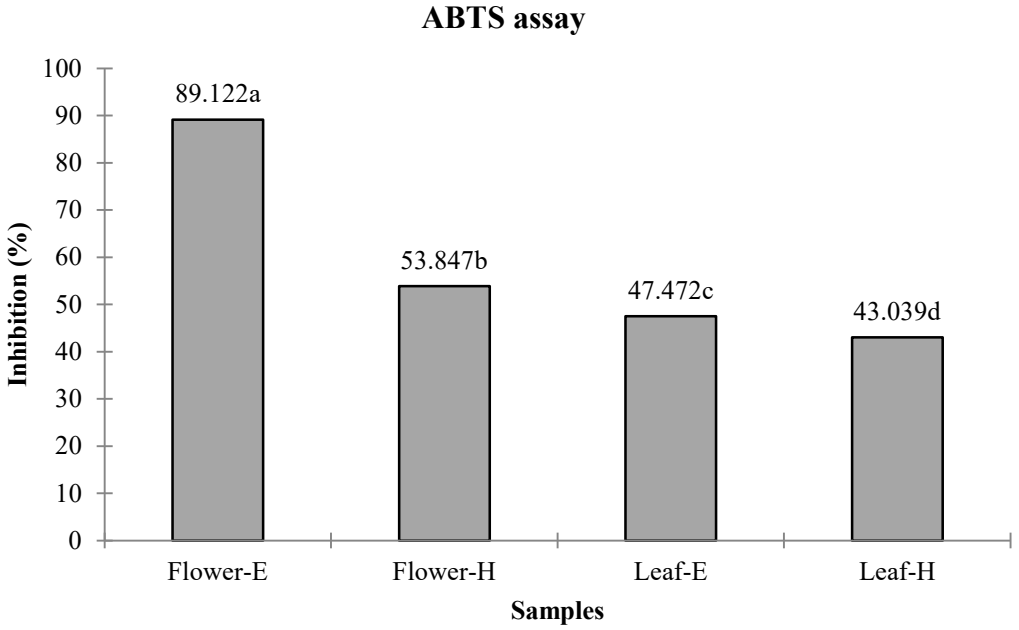


**Figure 2. Antioxidant effectiveness of leaf and flower of *H. scabrum* extracts using ABTS method. Ethanolic extract and H: Aqueous extract**

**Reducing power assay**

In this experiment, the more antioxidant compounds convert the oxidation form of iron ($Fe^{+3}$) in ferric chloride to ferrous ($Fe^{+2}$). In the reducing power assay, a significant difference observed among the extracts collected from the leaf and flower of *H. scabrum* (Figure 3). Maximum reducing power capacity was detected by the aqueous leaf extract, while the minimum value of reducing power efficiency was recorded by ethanolic leaf extract. The outcomes of this investigation presented that the reducing power of the ethanol fraction was less than water fraction, meaning that during the fractionation process an increase in the antioxidant activity occurred. Furthermore, the reducing power ability of the leaf extract was significantly higher than the flower extract.

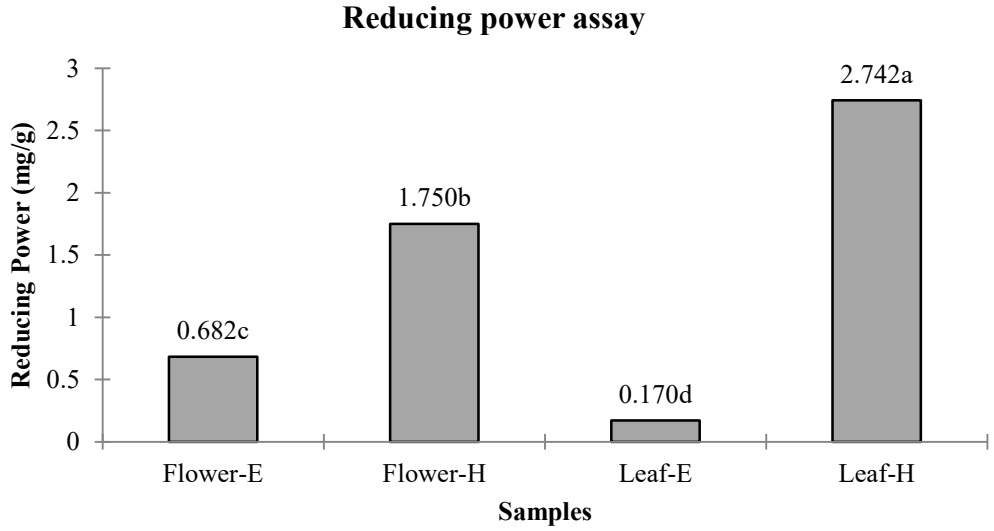


**Figure 3. Reducing power ability of different solvent used for extraction in leaf and flower of *H. scabrum.* Ethanolic extract and H: Aqueous extract**

**Total antioxidant capacity**

The data of Figure 4 demonstrates the total antioxidant capacity obtained through the phosphomolybdenum assay for each extract in comparison with that of ascorbic acid used as references. Significantly ($p \leq 0.05$), there were differences between the total antioxidant capacity of the flower and leaf extracts. The results in Figure 4 indicated that, the flower extracts exposed the highest total antioxidant capacity. The aqueous extract of flower displayed high amount of total antioxidant ability (3.005 mg TAE $g^{-1}$ extract) followed by the flower ethanolic extract (2.886 mg TAE $g^{-1}$ extract) and leaf aqueous extract (2.641 mg TAE $g^{-1}$ extract), which are not significant statistically. In addition, the minimum amount of total antioxidant capacity was registered by the leaf ethanolic extract.

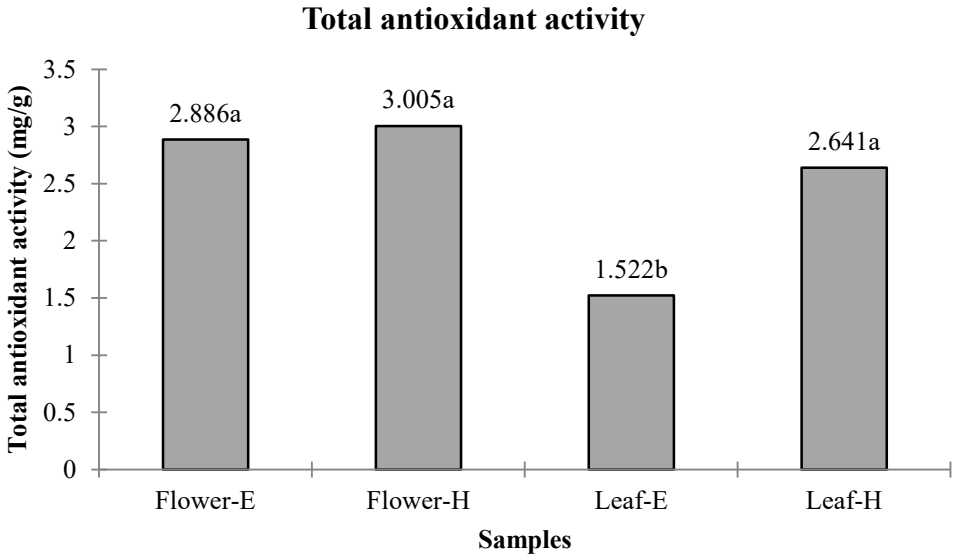


**Figure 4. Total antioxidant capacity of *H. scabrum* aqueous extract and ethanolic extract. The extracts having the different letter show significant differences ($p < 0.05$) according to Duncan statistical test. Ethanolic extract and H: Aqueous extract.**

**Correlations between the phytochemical contents and antioxidant capacities**

The antioxidant activity of an extract is often linked with their redo features, which allow them to act as reducing agents. Phenolic component constitutes one of the major classes of phytochemicals performing as free radical scavengers. The relationship between the results of the antioxidant assays and phytochemical contents of *H. scabrum* extracts were calculated using linear regression analysis and multivariate analysis (Table 2 and Figure 5). Total phenolic content content showed a significant and strong positive correlation with total antioxidant activity ($r = 0.950$, $P \leq 0.05$), and a moderate negative relationship with DPPH inhibition ($r= -0.710$, $P \leq 0.05$). Although, total flavonoid content showed significant and positive linkage with DPPH antioxidant activity ($r = 0.850$, $P \leq 0.05$), whereas total saponin content disclosed positive, strong relationship with the inhibition percentage of ABTS assay ($r = 0.965$, $P \leq 0.05$). These results indicated that the accumulations of total phenolic, flavonoid and saponin compounds in *H. scabrum* extracts, gave the antioxidant properties in *H. scabrum* extracts as shown by DDPH, ABTS and TAC analysis.

The principal component analysis (PCA) is a multivariate tool used for decreasing the dimensionality of a data set. The data of phytochemical contents and antioxidant activities was subjected to multivariate analysis, to determine the possibility grouping of different data collected from the contents of compounds and antioxidant assay. The variances explained by component were labelled in Figure 5. The two firs components recorded 92.49%. The first component accounts for 59.58% of the total variance and the second for 31.92%. The cumulative variance is of 86.91%; it indicates that the different extracts were well differed by their phytochemical amounts and antioxidant properties. It can be seen from Figure 5 that most of variables (total phenolics, flavonoïd and saponin contents,

DPPH radical, ABTS activity, reducing power and total antioxidant potential) display a high association with the first component, while the total tannin content was linked with the second factor. This result demonstrated that the total tannin content in *H. scabrum* was not a good indicator of the antioxidant efficiency. In addition, the PCA plot showed the ethanolic flower extract recorded the the highest value of total flavonoid and saponin contents and antioxidant activity measured with DDPH and ABTS methods.

**Table 2. Correlation coefficients between the contents of substances and antioxidant efficiency.**

| Variables | TPC | TFC | TSC | TTC | ABTS | DPPH | RP | TAC |
|---|---|---|---|---|---|---|---|---|
| TPC | **1** | | | | | | | |
| TFC | -0.527 | **1** | | | | | | |
| TSC | 0.028 | **0.793** | **1** | | | | | |
| TTC | **0.724** | **-0.799** | -0.360 | **1** | | | | |
| ABTS | 0.248 | 0.614 | **0.965** | -0.115 | **1** | | | |
| DPPH | **-0.710** | **0.850** | 0.605 | -0.617 | 0.442 | **1** | | |
| RP | 0.644 | -0.683 | -0.541 | 0.413 | -0.427 | **-0.933** | **1** | |
| TAC | **0.950** | -0.375 | 0.203 | 0.584 | 0.405 | -0.582 | 0.547 | **1** |

Values in bold are different from 0 with a significance level alpha=0.05

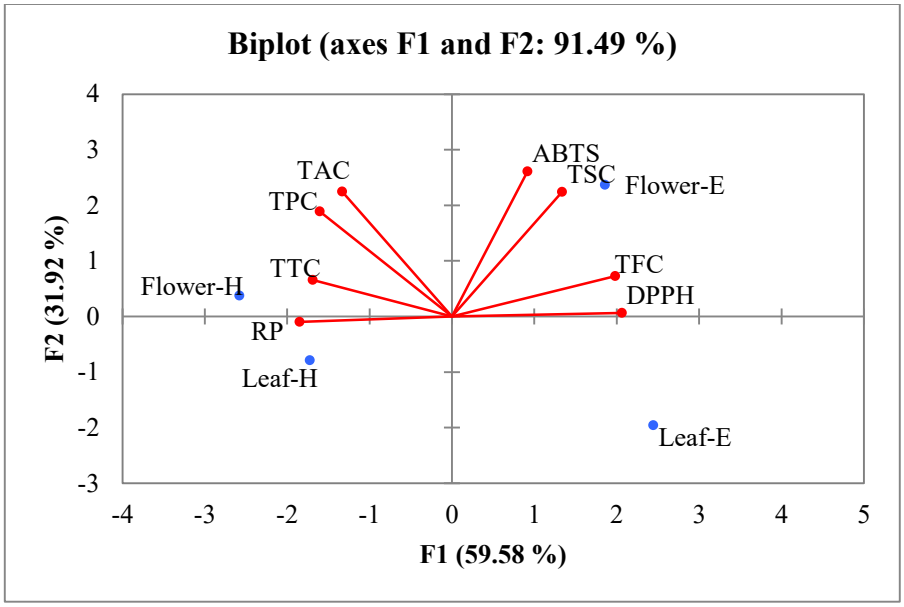


**Figure 5. PCA plot showing the distribution of phytochemical contents and antioxidant methods.**

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## تقدير المحتوى من المركبات الفعالة والفعالية المضادة للأكسدة لمستخلص اوراق وازهار نبات *Hypericum scabrum*

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**المستخلص**

تم تحليل المستخلص الكحولي والمائي لأوراق وازهار نبات *H. scabrum* لغرض دراسة محتواها من المركبات الفعالة وقابليتها المضادة للأكسدة. تم تقدير القابلية المضادة للأكسدة باستخدام أربع طرق، بما في ذلكDPPH، فحص إزالة اللونABTS ، تقليل من القوة والقابلية المضادة للأكسدة الكلية. أظهرت النتائج احتواء المستخلص الكحولي للأزهار على كميات أعلى من الفلافونويدات (4.674 ملغم\غم) ومن السابونين (29.752 ملغم\غم)، في حين احتوى المستخلص المائي للأزهار على أقل معدل للفينولات الكلية ($1.223\ mg.\ g^{-1}$) . كما بينت النتائج تفوق المستخلص الكحولي للأزهار على بقية المستخلصات في قابلية المضادة للأكسدة باستعمال طريقة DPPH وABTS . كما لوحظ علاقة عالية المعنوية من الفينولات الكلية والفعالية المضاد للأكسدة، وبين المحتوى الفلافونويدات الكلي وتثبيط بواسطة DPPH. كشف تحليل المكون الاساسي(PCA) عن وجود 91.49 من التباين الكلي الذي يشير إلى تتميز المستخلصات المختلفة بمحتواها من المواد الفعالة وفعاليتها المضادة للأكسدة.

**الكلمات المفتاحية: المركبات الفعالة، مضادات الاكسدة، *Hypericum scabrum*.**