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Research Article

TOTAL PHENOLIC, TOTAL FLAVONOID CONTENTS AND RADICAL SCAVENGING ACTIVITIES OF 10 ARABIAN HERBS AND SPICES

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ABSTRACT

The current study attempted to investigate the antioxidant activities of 10 Arabian herbs and spices namely, *A. obesum* flower, *T. foenum-graecum* leaves, *S. officinalis* leaves, *Z. piperitum* seeds, *A.vera* gel, *I. verum* seeds, *A. corrorima* fruits, *N. sativa* seeds, *L.sativum* seeds, and *Z. spina-christi* leaves by determining their total phenolic, total flavonoid and Trolox equivalent antioxidant capacity. The mean values of the total phenolic content were 1889.66, 789.63, 235.33, 1264.85, 5477.53, 693, 62.37, 166.05, 602.25, and 938.27 (mg GAE/100g) in *A. obesum , T. foenum-graecum, S. officinalis, Z. piperitum , A. vera , I. verum , A. corrorima, N. sativa, L. sativum,* and *Z. spinachristi* respectively, whereas the mean values of the total flavonoid content were 553.52 , 598.82, 178.67, 434.4, 1958.27, 112.13, 4.33, 36.87, 61.85, and 249.56 (mg QEA / 100g) respectively. The mean values of Trolox equivalent antioxidant capacity (TEAC) were 6328.51, 1273.2, 579.07, 4357.45, 11642.56, 1842.38 , 158.77, 511.15 , 910.22 , 3293.33 (µmole /100 g) in *A. obesum, T. foenum-graecum, S. officinalis, Z. piperitum , A. vera, I. verum , A. corrorima, N. sativa , L. sativum,* and *Z. spina-christi,* respectively. Our findings showed that *A. vera* gel and *A. obesum* have a high total phenolic content, also the two plants possess a considerable content of total flavonoid and they also have a high levels of TEAC radical-scavenging activity compared with the current studied plants. It will be useful to further analyze *A. vera* gel and *A. obesum* flower in order to separate and identify their possible protective roles against diabetes mellitus and hyperlipidemia.

Keywords: Aloe vera gel, *Adenium obesum* flower, Total phenolic, total flavonoid, Trolox equivalent antioxidant activity.

INTRODUCTION

The traditional medicine becomes a major area of scientific research and the widespread use of traditional herbs, spices, and medicinal plants has been traced to the occurrence of natural products with medicinal properties, the antioxidant activities of their constituents, and a wide range of their phenolic compounds. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers, and metal chelators[1,2,3]. The overproduction of free radicals, as a byproduct in metabolic processes, can cause oxidative damage to biomolecules and could lead to many chronic diseases. Several reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases such as diabetes, cancer, atherosclerosis, aging, and other degenerative diseases in humans[4-7]. The aim of this study is to establish the total phenol, total flavonid contents and the antioxidant capacity of ten of the most popular Arabian and Chinese medicinal plants.

MATERIALS AND METHODS

Chemicals and reagents

Methanol and other chemicals were of analytical grade and were obtained from BDH (Poole, UK). Gallic acid, Folin-Ciocalteu reagent, quercetin, 2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS),and potassium persulfate were purchased from Sigma-Aldrich(St. Louis, MO,USA). Trolox (6-hydroxy-2, 5, 7, 8- tetramethylchroman-2- carboxylic acid) was obtained from Fluka Chemie AG (Buchs, Switzerland).

Plant material and extraction

Adenium obesum (Apocynaceae) flower was obtained from Yemen, *Trigonella foenum-graecum* (Leguminosae) leaves, *Salvia officinalis* (Lamiaceae) leaves, *Aloe vera* (Aloeaceae) gel, *Illicium verum* (Schisandraceae) (Star anise) seeds, *Aframomum corrorima* fruit (Zingiberaceae), *Nigella sativa* (Ranunculaceae) seeds, *Lepidium sativum* (Brassicaceae) seeds, were obtained from the local market, Jeddah , the western region of Kingdom of Saudi Arabia. *Ziziphus spina-christi* (Rhamnaceae) leaves were obtained from Al-Madina

Al-Monawara, the western region of Kingdom Saudi Arabia. *Zanthoxylum piperitum* (Rutaceae) fruit was obtained from the south west region of China.

Plants were identified by staff members of the Botany Department, College of Science, King Saud University, Saudi Arabia. Voucher specimens were deposited at the herbarium of the Botany Department. Powder extracts were obtained by use of a mill and they were stored in amber bottles to prevent degradation. The methanol extract of each plant powder was obtained by dissolving 2g of each plant powder in 20 ml methanol-water(4:1v/v) at room temperature overnight using an orbital shaker. Filtrates were collected and residues were extracted again with 20 ml solvent. Appropriate filtrates were pooled and centrifuged at 5000 rpm for 10 min, and the supernatants were concentrated under reduced pressure at 40ºC using a rotary evaporator. After solvent evaporation, residues of each extract were dissolved in distilled water and the final volume was recorded. A serial dilutions of 10, 100, 1000, and 5000 were made for each plant extract and stored at −20 ºC until analysis.

Determination of total phenol

The amount of total phenol content was determined by Folin-Ciocalteu's reagent method[8]. About 0.5 ml of each diluted extract and 0.1 ml Folin-Ciocalteu's reagent were mixed and incubated at room temperature for 15 min. Then, 2.5 ml of 7% sodium carbonate solution was added, the tubes were incubated for 30 min at room temperature and the absorbance was measured at 760 nm. Gallic acid standard curve was constructed (0 -50 µg). Total phenol contents of extracts were expressed as mg gallic acid equivalents (GAE)/100 g sample.

Determination of total flavonoid

The total flavonoid content was determined by aluminum chloride colorimetric method[9]. Briefly, 0.5ml of each diluted extract was mixed with 1.5 ml of 95% ethanol , 0.5 ml of 1.2% aluminum chloride , 0.5 ml of 120 mM potassium acetate were added in order. After incubation at room temperature for 30 min, the absorbance was measured at 415 nm against reagent blank. Quercetin standard curve was constructed (0- 50 µg). The total flavonoid content in samples was expressed as milligram quercetin antioxidant equivalents (QAE)/100g sample.

Radical cation ABTS scavenging activity

The ABTS free radical decolorization assay was performed as described before[10]ABTS radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation at room temperature in dark for 16 hr. The ABTS solution was diluted with 80% ethanol to an absorbance of 0.700 ± 0.050 at 734 nm. 0.1 ml of each diluted extract and 3.9 ml ABTS solution were mixed thoroughly. The mixture was allowed to stand at room temperature for 6 min and the absorbance was immediately recorded at 734 nm. Trolox standard curve (0–15 µM) in 80% ethanol was prepared. Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC, µmol Trolox equivalents per g sample).

Data analysis

The data are presented as means ± standard deviation. All samples were analyzed in three replications.

RESULTS AND DISCUSSION

The standard curve of total phenol using gallic acid as standard is presented in Fig.1. The total phenolic content of the aqueous extracts were 1889.66, 789.63, 235.33, 1264.85, 5477.53, 693, 62.37, 166.05, 602.25, and 938.27 (mg GAE/100g) in *A. obesum, T. foenumgraecum, S. officinalis, Z. piperitum , A. vera , I. verum , A. corrorima, N. sativa , L. sativum ,* and *Z. spina-christi* respectively (Fig.2). *A. vera* showed the highest phenolic content (5477.53 mg GAE / 100g). Total phenolic content of A. vera extracted with 75 mM phosphate buffer p H 7.5 is 0.23 mg GAE / g of fresh weight[11]. *A. obesum* flower also showed considerable concentration of total phenolics (1889.66 mg GAE / 100 g). The total phenolic content of *A. obesum* flower as ascorbic acid equivalent per g (AAE/g) was 30.30 mg AAE/g[12]. Another report[13]concluded that *A. obesum* extracts collected from Yemen are rich sources of anthocyanins and possess a significant antioxidant activity. The total phenolic content of *Z. piperitum* leaves (1264.85 mg GAE / 100 g). Jeong et al.[14] reported the presence of a high level of total phenolics, particularly quercitrin, in *Z. piperitum* leaves ranging from 17.2 – 2425 mg Quercitrin /100 g when extracted by silica-gel open column chromatography and these phenolics could reduce the risk of neurodegenerative disorders. The polyphenol content of the methanol extract of Japanese pepper fruit was 6.0% (w/w) quercitrin equivalent using Folin Ciocalteu reagent[15]. Our obtained result for total phenolics content of *T. foenum-graecum* leaves (789.63 mg GAE / 100 g) is comparable with the report of [16] who stated that the total phenolic content of fenugreek seed extract is between 13.08 – 14.23 mg GAE/g . In complementary medicine, *Z. spina-christi* leaves are used for the treatment of dysentery, leprosy, parasitic infections, and gastro internal disorder. The total phenolics content of *Z. spinachristi* leaves (938.27 mg GAE / 100 g), however, the concentration of the same parameter in the fruit was reported to be 1644.00 ±3.20 mg GAE / 100 g[17.] The total phenolic content of *L. sativum* and *I. verum* were 602.25 and 693.00 mg GAE / 100 g respectively. Our findings of *I. verum* total phenolics content is coincided with the report of[18] which stated that the total polyphenols of ambient temperature water extract of Star Anise is 7.31±0.61 mg GAE / g , whereas the total phenolic content of *L. sativum* is in a full agreement with the concentration range between 6.33 – 7.40 mg GAE / g other report[19]. The determined total phenolic content of *S. officinalis* in our report is 235.33 mg GAE / 100 g, however[20] reported that Sage total phenols concentration is 5.95±2.65 mg GAE/g of dried extract, this discrepancy could be attributed to the quality of the herb.The total phenolic content of *A. corrorima* fruit obtained in this investigation 62.37 mg GAE/100 g, is in contrast to the concentration of the same parameter in the plant seeds and pods stated[21]. Our finding regarding the total phenolic content of *N. sativa* (166.05 mg GAE/100g) is consistent with the report of Souri et al.[22] who stated that the phenol content of *N. sativa* is 122.67 ± 3.03 mg GAE/100 g. The standard curve of total flavonoid using quercetin as standard is presented in Fig.3. the total flavonoid content of the aqueous extracts were 553.52 ,598.82,178.67,434.4, 1958.27, 112.13, 4.33, 36.87,

61.85, and 249.56 (mg QEA / 100g) in *A. obesum, T. foenum-graecum, S. officinalis, Z. piperitum, A. vera, I. verum, A. corrorima, N. sativa, L. sativum* and *Z. spina-christi,* respectively (Fig.4). *A. vera* showed the highest content of total flavonoid 1958.27 mg QE / 100g, whereas the lowest total flavonoid content was noticed in *A. corrorima* (4.33 mg QE / 100g). The antioxidants of the ethanolic extract of *A. vera* reported to have an antihyperglycemic effect through the prevention of excessive production of free radicals and by decreasing activities of antioxidant enzymes[23]. *T. foenum-graecum* leaves also contained a considerable amount of total flvonoid (598.82 mg QE / 100g), whereas, the metabolic extract of the plant seed reported to have a total flavonoid content(as Catechin) of 20.8 mg CE/100g[24]. Our obtained result for the total flavonoid content of *Zanthoxylum piperitum* fruit was 434.4 mg QE/100g. The total flavonoid content in *Zanthoxylum alatum* fruit, another species of the plant, reported to be 6.66 ± 0.06 mg QE /g[25]. The fruit of *Z. spina-christi* grown in Oman has a total flavonoid content of 47 mg CE/100 g[17], however, our findings revealed that the leaves of the same plant has a total flavonoid content of 249.56 mg QE/100 g which is five times higher than that reported by17 Singh et al. [17].

The standard curve of trolox for determining the radical cation ABTS scavenging activity as trolox equivalent antioxidant capacity (TEAC) is presented in Fig.5. The TEAC of the aqueous extracts were 6328.51, 1273.2 , 579.07, 4357.45, 11642.56, 1842.38, 158.77, 511.15, 910.22 , 3293.33 (µmole /100 g) in *A. obesum , T. foenum-graecum, S. officinalis, Z. piperitum, A. vera, I. verum, A. corrorima, N. sativa, L. sativum, and Z. spina-christi* respectively (Fig. 6). Anilakumar et al.[26] attributed the protective effect of *A. vera* gel extract against azoxymethane – induced oxidative stress in rats to the antioxidant potency of the plant. From this view, our obtained findings that *A. vera* gel extract has a high TEAC (11642.56 µmole/100 g) is in a full agreement with the report of [26]. *A. obesum* also showed a considerable level of TEAC (6328.51µmole /100 g), however[13] reported that 100% methanol extract of *A. obesum* has the best free radical scavenging activity due to its high content of anthocyanins. *Z. piperitum* and *Z.spina-christi* they were also showed a good levels of TEAC, 4357.45 and 3293.33 µmole /100 g respectively (Fig. 6).

Trolox equivalent antioxidant capacity (TEAC) is positively correlated to total phenolic and total flavonoid contents of the investigated plants, the correlation coefficients (R^2) were 0.9349 and 0.8365 respectively (Fig.7a and 7b). Some reports referred to this linear positive correlation between ABTS assay antioxidant activity with the total phenolic and the total flavonoid content of other herbal extracts [27, 28, 29].

CONCLUSION

In the current study, the total phenolic, total flavonoid, and TEAC radical-scavenging activity of ten Arabian herbs and spices were determined. The results showed that *A. vera* gel and *A. obesum* have a high total phenolic content, also the two plants possess a considerable content of total flavonoid. *T. foenum-graecum*, as well, has a high content of total flavonoid. Once again, *A.vera* gel and *A. obesum* have high levels of TEAC radical-scavenging activity compared with the current studied herbs and spices. Our future work will focus on the use of sophisticated techniques such as chemiluminescence and high performance liquid chromatography for the separation of the active principles of *A. vera* gel and *A. obesum* extracts and identification of their possible protective roles against diabetes mellitus and hyperlipidemia.

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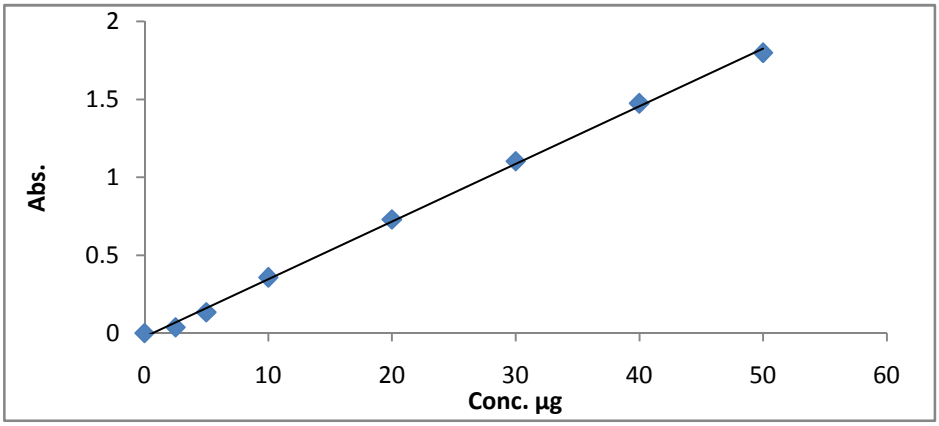

Figure 1: Standard curve of Gallic acid

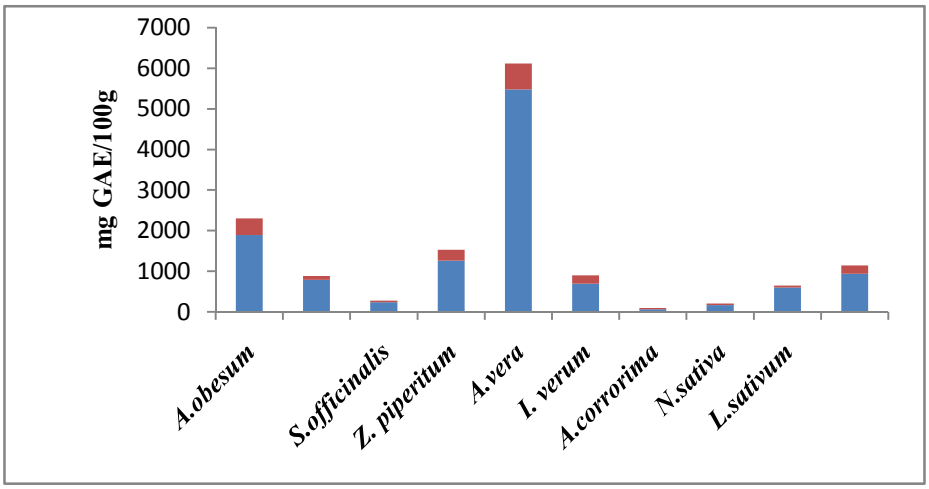

Figure 2: Total phenol content as mg gallic acid (GAE) /100g of *A. obesum*, *T. foenum-graecum* , *S.officinalis* , *Z. piperitum* , *A.vera* ,*I. verum, A.corrorima, N.sativa* ,*L.sativum*, and *Z.spina-christi*. Data are expressed as means ± standard deviation and calculated from three determinations.

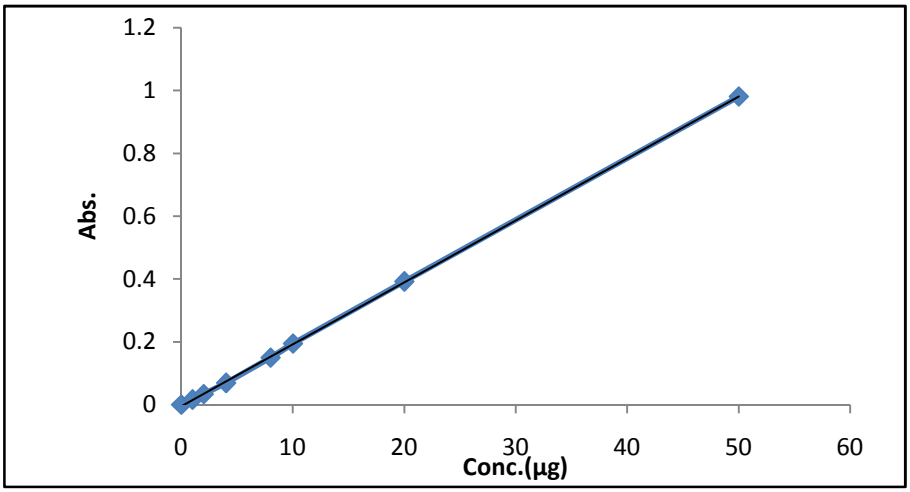

Figure 3: Standard curve of Quercetin

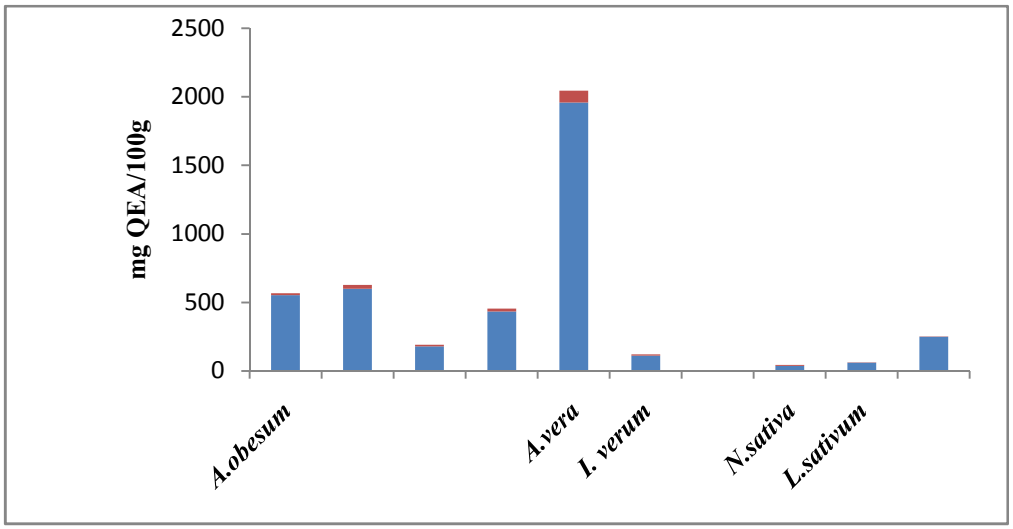

Figure 4: Total flavonoid content as mg Quercetin(QEA) /100g of *A. obesum*, *T. foenum-graecum* , *S.officinalis* , *Z. piperitum* , *A.vera* ,*I. verum, A.corrorima, N.sativa* ,*L.sativum*, and *Z.spina-christi*
Data are expressed as means ± standard deviation and calculated from three determinations.

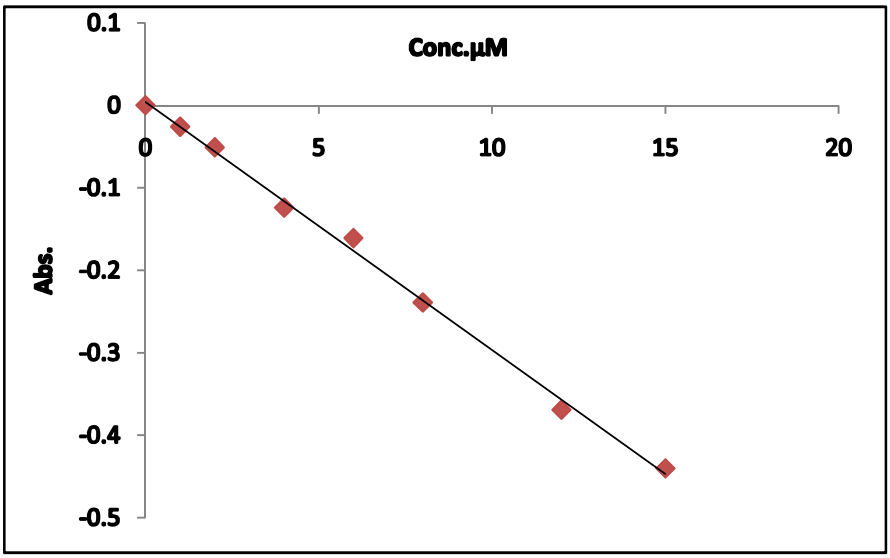

Figure 5: Standard curve of Trolox

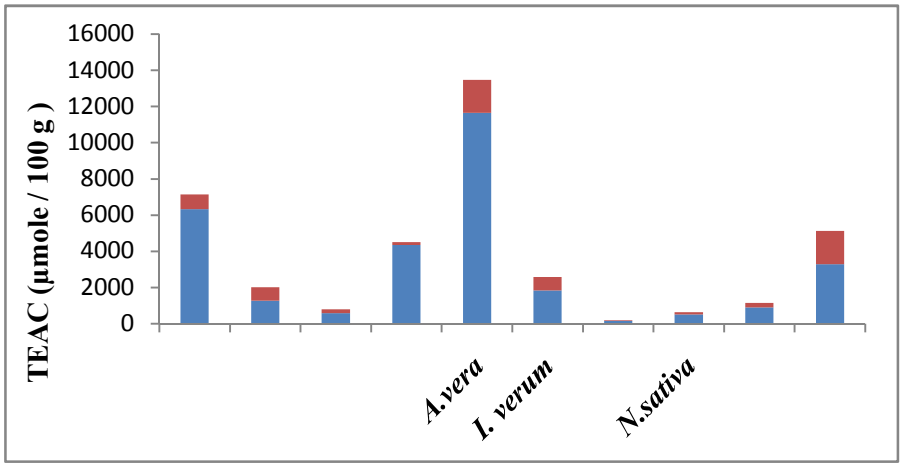

Figure 6: Trolox equivalent antioxidant capacity as µmol TEAC/100 g) of *A. obesum*, *T. foenum-graecum* , *S.officinalis* , *Z. piperitum* , *A.vera* ,*I. verum*, *A.corrorima*, *N.sativa* ,*L.sativum*, and *Z.spina-christi*
Data are expressed as means ± standard deviation and calculated from three determinations

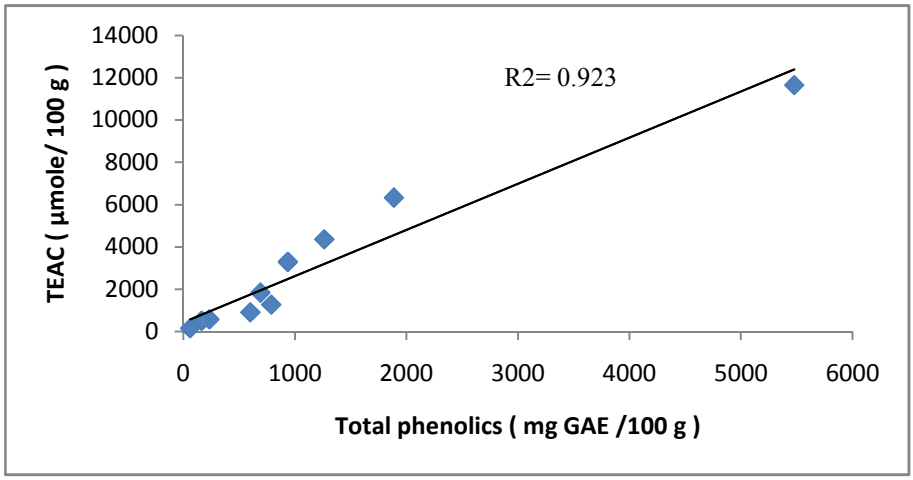

Figure 7a: Correlation between the total phenolic content as gallic acid equivalents (GAE) and the Trolox equivalent antioxidant capacity (TEAC) of *A. obesum*, *T. foenum-graecum* , *S.officinalis* , *Z. piperitum* , *A.vera* ,*I. verum*, *A.corrorima*, *N.sativa* ,*L.sativum*, and *Z.spina-christi*

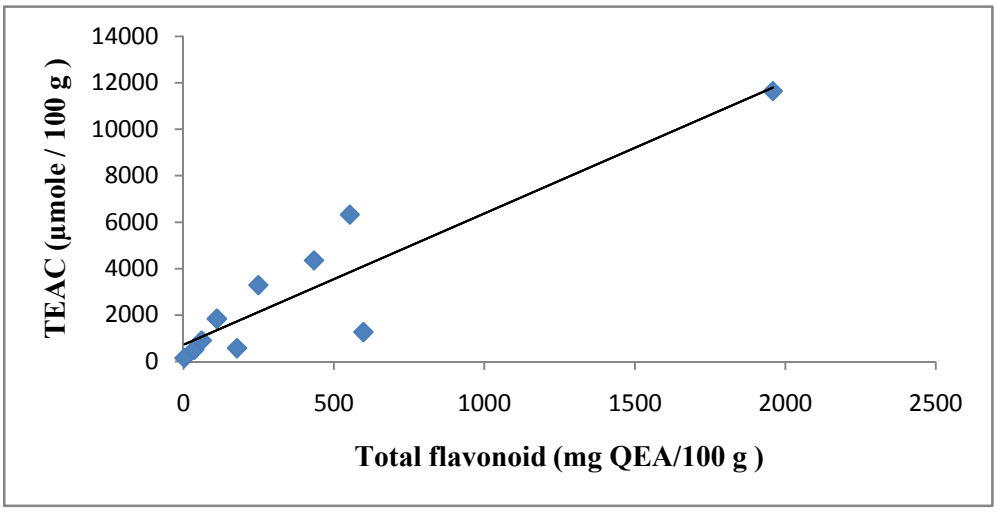

Figure 7b: Correlation between the total flavonoid content as quercetin antioxidant equivalents (QAE) and the Trolox equivalent antioxidant capacity (TEAC) of *A. obesum*, *T. foenum-graecum* , *S.officinalis* , *Z. piperitum* , *A.vera* ,*I. verum, A.corrorima, N.sativa* ,*L.sativum*, and *Z.spina-christi*

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