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Antioxidant, and Antimicrobial Activities of Phenolic and Flavonoid Rich Medicinal Plants (*Fritillaria zagrica* and *Tulipa kurdica*) Bulbs Collected in Kurdistan Region of Iraq

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A B S T R A C T

Fritillaria zagrica and *Tulipa kurdica* had been used as traditional herbal remedies since antiquity to treat human diseases in the Kurdistan region of Iraq. This is the first report and conceive to analyze these two medicinal plants based. Methanol, 80% ethanol and aqueous extracts of two medicinal plants (*Fritillaria zagrcia* and *Tulipa kurdica*) were evaluated for their antibacterial activity and antifungal activities. We have quantified the total phenolic (TPC) and flavonoid (TFC) contents and their relation to antioxidants (ABTS) [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) and 2,2′-diphenyl-1 picryl hydrazyl (DPPH)scavenging free radicals in a dose dependent method. Results showed that *F. zagrica* and *T. kurdica* bulb extractions by using different solvents exhibited strong Antimicrobial activities against selected bacterial and fungal strains except *C. guilliermondii ATCC 6260*. Aqueous bulb extract of *F. zagrica* and *T. kurdica* showed better antibacterial activity compared to other solvent extracts. Methanol *F. zagrica* bulb extracts contain significantly higher phenolic content than *T. kurdica*. The TPC and TFC contents were varied between the plant bulb extracts and the DPPH and ABTS scavenging activities were exhibited to be significantly correlated with the amount of TPC and TFC.

1. INTRODUCTION

Kurdistan is a closed and stored pool as a buried treasury industrial pharmacy. There are a large number of medicinal plants used in folk medicine by the traditional herbalists to treat varieties of human ailments. Among them, *Fritillaria zagrica* and *Tulipa kurdica* had been priced for their local traditional medicinal resources.

Fritillaria zagrica Stapf. is a species very closely allied to *F. tulipifolia* and *F. armena*. Their flowers dark lurid untesselated, unchequered with a thick bloom outside, purple glaucous outside and at the tips of the petals there is always a bright yellow spot (Weathers, 1911, Ruksans, 2007). *F. zagrica Stapf.* belongs to the *Fritillaria*, which is a genus of over 160 species of bulbous plants within the monocot family Liliaceae, native to temperate regions of the Northern Hemisphere (Liu et al., 2012, Hao et al., 2015, Kiani et al., 2015). The species of the *Fritillaria* genus are distributed especially in the Mediterranean regions and eastern parts of Anatolia and Thrace, the Black Sea, and Central Anatolia in Turkey, and in Iran, Iraq, Syria, Afghanistan, Russia, Lebanon, Palestine, Jordan, Pakistan, China, Japan, Greece, Bulgaria, Italy, Spain, Portugal, North Africa, and California and Oregon in the USA (Tekşen and Aytaç, 2011). *Fritillaria* L.

has been commonly used in traditional Chinese medicine for thousands of years, contain many pharmaceutically active constituents (Hao et al., 2013).

Many *Fritillaria* species have long been exploited as the natural source of the widely used traditional medicine 'bulbus Fritillaria' (*i.e.*, dry bulbs or a decoction of *Fritillaria* species; 'Beimu' in Chinese) that has long been used as one of the most important antitussive, expectorant, and antihypertensive drugs and treat tumors, hemoptysis and deficiency of milk in traditional Chinese medicine (Li et al., 2006, Li et al., 2013, Matsuo et al., 2013).

Similarly, *T. kurdica* belongs to *Tulipa L.* (Liliaceae), which is a genus of about 100 species of bulbous monocots (Bryan, 2002, Bryan, 2005, Zonneveld, 2009) naturally occurring from southwestern Europe and North Africa, the Middle East to Central Asia (Christenhusz et al., 2013). *T. kurdica* closely to *T. humilis* which has stems 4-6 inch and flowers brick red or orange red with the basal blotch black, late spring (Bryan, 2005). From a medicinal and industrial viewpoint, *Tulipa* and *Fritillaria* are two of the most important genera in Liliaceae family due to their relatively rich pharmacological history (Li et al., 2006).

Likewise, the bulbs of *F. zagrica* and *T. kurdica* have been traditionally used in clinic as herbal remedies by Kurdish people who are living in the mountains of Kurdistan Region-Iraq, due to their positive potent therapeutic effectivities during lesion treating, wound healing, preventing and reducing the pain and removing the excessive tumor mass (lump) beneath the skin of breast, armpit and underarm with discharging abscess.

The medicinal values of the plants depend on the presence of certain chemical substances called secondary metabolites, involving alkaloids, tannins, flavonoids, terpenes and phenolic compounds that are involved in antioxidant and free radical scavenging (Saniewski and Horbowicz, 2003, Sobia, 2011). Phenolic compounds are secondary plant metabolites that are found naturally in all plant materials, including plant based food products. These compounds are thought to be an integral part of human and animal diets. They represent the most important group of natural antioxidants (Huyut et al., 2017). Furthermore, as a result of high medicinal plants application in treating many diseases, now the plants are also useful as novel antimicrobial agents (Khan et al., 2018).

In particular, reactive oxygen species (ROS), such as hydroxyl radicals, superoxide anions, and hydrogen peroxide, are frequently generated spontaneously in the living cell during metabolism and play an important role in cell signalization (Liu et al., 2012). However, excessive amount of ROS can induce oxidative stress, resulting in significant damage to cell structures and macromolecules, including proteins, lipids, and nucleic acids. As it is well known, ROS also contributes in development of cancer, inflammation, diabetes, aging, inflammation and cardiovascular disease (Marvibaigi et al., 2016, Ismail et al., 2017).

In this context, the antibacterial and antioxidant properties of various medicinal plants are being investigated throughout the world because of the toxicological concerns associated with the synthetic antioxidants and preservatives (Baba and Malik, 2014).

However, to the best of our knowledge, there is no scientific work on antimicrobial and antioxidant activities of *F. zagrica and T. kurdica* medicinal plants. Therefore, the aim of this study to determine the total phenolic-, flavonoid- contents and to elucidate the antioxidant properties and antimicrobial activities of bulb extracts of both *F. zagrica*

and T. kurdica medicinal plants grown in Kurdistan region- Iraq.

2. MATERIALS AND METHODS

2.1. MATERIALS

2.1.1 Plant Material Collection:

Fritillaria zagrica and *T. kurdica* were collected in April 2014 from Zine-Asterokan mountain near to Karokh mountain and Weza village near Choman district - Erbil / Kurdistan region from northern Iraq, respectively. The plants were classified and identified by botanists Prof. Dr. Abdul Hussain Al Khayat and Mrs. Bnar Khalid Bakr according to the most common classification key. The voucher specimens, accession number (0007627) for *F. zagrica* and (0007628) for *T. kurdica* plants were deposited at the Herbarium of Department of Biology, College of Science, Salahaddin University, Kurdistan-Iraq. The plants raw materials (bulbs) were washed and air-dried under shade at room temperature (20-25^{o}C). After drying, the bulbs' plants were grounded into fine powder using a laboratory grinding mill and sieved with 710 um sieve, to provide homogeneous powder for analyzing. Powdered materials were stored in bottles in a dark room temperature and then used.

2.1. .2 Preparation of Freeze-Dried Bulb Extracts

The powdered *F. zagrica* and *T. kurdica* plants bulb materials (100 g) were carried out and macerated with 1 L of extracting solvents (99.9 % absolute methanol and 80% ethanol) and water in the beakers covered by aluminum foil and incubated in Ultrasonic bath at room temperature for two hours. The solvents were drained out after 4 h and replaced with fresh one. This procedure was repeated thrice. The extracts were then separated from the sample residues by filtration through Whatman No.1 filter papers. The resultant bulb extracts were concentrated in a rotary evaporator in a water bath at 40 °C until the crudes solid extracts were obtained, which were then freeze-dried for completing solvents removal. Finally, the obtained bulb extracts were weighed, their yields were calculated, and stored at -20 °C in sealed tubes until used for further analysis. The methanol, 80 % ethanol and water extracts' yields were 7.76, 2.97, 2.60 % for *F. zagrica* bulb extracts while the methanol, 80 % ethanol and water yields were 7.40, 4.70, 3.57 % for *T. kurdica* bulb extracts respectively.

2.2 Chemicals and Microorganisms

All chemicals and reagents used in the study including solvents were of analytical grade. Methanol and Ethanol were obtained from Score Scientific SDN BHD. 1,1-Diphenyl- 2-picryl hydrazine (DPPH), 2, 2'-azino bis-(3-ethyl benzo thiazoline-6-sulphonic acid) (ABTS), quercetin, catechin, Folin-Ciocalteu's phenol reagent, Difco™ nutrient broth, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman- 2-carboxylic acid (Trolox), sodium nitrite, sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), aluminum chloride ($AlCl_3$), potassium persulfate ($K_2S_2O_8$), ethylenediaminetetraacetic acid (EDTA), and ascorbic acid were purchased from Sigma Aldrich Chemicals (USA). Dimethyl sulfoxide (DMSO) was obtained from Fisher Scientific (USA).

The standard bacterial strains of the Gram negative bacteria *Escherichia coli* (*ATCC 25922*), *Pseudomonas aeruginosa* (*ATCC 27853*), *Acinetobacter baumannii* (*ATCC 19606*) and of the Gram positive bacteria *Staphylococcus aureus* (*ATCC 25923*) and *Bacillus subtilis* (*ATCC 6633*) and pathogenic fungal strains, *Candida tropicalis* (*ATCC 13803*), *Candida albicans* (*ATCC*

10231), *Candida guilliermondii* (*ATCC 6260*) were used and obtained from the Department of Biology, College of Science, Salahaddin University, Erbil/ Kurdistan-Iraq. A standard antibiotic (amikacin) was used as positive control.

2.3 Assay of Antibacterial Activity against Pathogenic Bacterial Strains

The antibacterial activities have been checked against Gram-negative *E. coli* (*ATCC 25922*), *P. aeruginosa* (*ATCC 27853*), *A. baumannii* (*ATCC 19606*) and Gram-positive *S. aureus* by using a modified Kirby-Bauer disk diffusion method. Aliquots of bacterial suspension (100 uL) were spread on Difco™ nutrient agar containing testing microorganism with optical density of 0.7 at 595 nm. Filter paper disks (8 mm) saturated with 20 uL of the prepared bulb extracts *F. zagrica* and *T. kurdica* (10 mg/ mL) in different solvents were placed on nutrient agar seeded with tested bacteria. The plates were incubated for 24 h at 37 ºC, and then the zones of growth inhibition were measured (Sharma et al., 2018). All the tests were done in triplicate and mean ± S.D. were calculated.

2.4 Assay of Antifungal Activity against Pathogenic Fungi

Sensitivity of *Candida spp.* were tested against bulb extracts of *F. zagrica* and *T. kurdica* (1 mg/mL) in methanol, 80% ethanol and aqueous solution by using filter paper disc diffusion method (agar disc diffusion method) the filter paper disc prepared by using ordinary office two-hole puncture, paper discs with approximate diameter of 6mm. were punched out one by one from a sheet of filter paper, the disks placed in vials, sterilized by oven and allowed to cool. Then sterilized discs (6 mm) were soaked in known concentration (1 mg/mL) of plant extracts; another filter paper disc was soaked with sterilized distilled water (SDW) used as negative control and DMSO as a solvent control, 50-100 discs were placed in small sterile air tight labeled containers and then allowed to dry for 2 hours. The sterile discs were placed in petri dishes. For antifungal activity, yeast suspension prepared from 24h colony by using phosphate buffer saline (PBS) in compare with standard control with concentration $41.5*10^6$ cell/mL of yeast suspension. Then 0.1 ml of yeast suspension was spread over sabouraud glucose agar medium (SGA), then incubated at 37^oC for 24-48h. Zones of inhibition were obtained by measurement of the radius from the center of the disc to the edge of the x inhibition of growth. Measurements were made from both sides of the slope and their average accepted (Al-Refai, 2006, Kamel et al., 2014).

2.5 Determination of Total Phenolic Content

The total phenolic content (TPC) quantification of *F. zagrica* and *T. kurdica* plants bulb extracts were achieved by Folin-Ciocalteu colorimetric method with slight modifications (Hatami et al., 2014, Marvibaigi et al., 2016). Briefly, 100 µl of extracts (1mg/ml diluted in distilled water) were mixed with 100 µl of 0.2 N Folin-Ciocalteu reagents. After 5 min with intermittent shaking, 80 µl of 7.5% sodium carbonate (Na_2CO_3) solution was added and incubated for 2h at room temperature. The absorbance (of the resulting blue color) was measured at 765 nm against the blank. The calibration curve was prepared using the standard gallic acid solution. All TPC determinations were carried out three times (n = 3) and the results were expressed in mg of Gallic acid equivalent (GAE/100 g of sample) (Marvibaigi et al., 2016, Ismail et al., 2017) as shown in figure 1.

2.6 Determination of Total Flavonoid Content (TFC)

The total flavonoid content estimation of *F. zagrica* and *T. kurdica* plants bulb extracts were measured spectrophotometrically by aluminum chloride colorimetric method, which is based on the formation of a flavonoid–aluminum complex with slightly modifications (Lin and Tang, 2007, Marvibaigi et al., 2016). Briefly, 200 µl of extract (1 mg/mL) was mixed with 12 µl 5% $NaNO_2$ and 12 µl 10% $AlCl_3$. After 5 min incubation at ambient room temperature, 80 µl of NaOH was added and re-incubated for 30 min. The absorbance of samples was read at 515 nm. The calibration curve was prepared using standard catechin solution. The TFC of extracts were expressed in mg of g catechin equivalent (CE/ 100 g of sample) as illustrated in figure 2. The analysis was performed in triplicate and mean values were reported.

2.7. Antioxidant Assay

2.7.1. DPPH Free Radical Scavenging Activity Assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl radical), is a dark-colored crystalline powder composed of stable free-radical molecules. The antioxidant activities were determined when using DPPH as a free radical because the use of $DPPH^{\bullet}$ provides an easy and rapid way to evaluate the antiradical activities of antioxidants (Brand-Williams et al., 1995). Hence DPPH has major application in laboratory research most notably in anti-oxidant assays (Bhandari et al., 2017). The antioxidant activity of *F. zagrica* and *T. kurdica* plants bulb extracts, on the basis of the scavenging activity of the stable DPPH free radical ($DPPH^{\bullet}$) in prepared 96 well plates, were performed following a previously described method with slight modifications (Iqbal et al., 2006, Akter et al., 2010). For each determination, the stock solution (1mg/ml) was diluted to a dilution series (156 µg-1000 µg/ml) with DMSO. An aliquot of each extract working concentration (20 µl) was mixed with methanolic solution of DPPH (200 µl, 0.06 mM). The mixtures were shaken vigorously and incubated at room temperature in the dark for 30 min. A control sample was placed under the same conditions. A color change from violet to yellow occurred during the reaction time. Sample absorbance was read at 517 nm using UV-VIS spectrophotometer and the percentage of free radical scavenging potential of the different extracts against $DPPH^{\bullet}$ was determined using the following equation:

DPPH radical scavenging activity (%) = [(Abs control- Abs sample) /Abs control] x100

DPPH solution was used as a control and ascorbic acid and quercetin were used as references standard. The percentage of DPPH scavenging versus concentration of samples was plotted. The antioxidant activity was expressed as median effective concentration (EC50) where the concentration caused 50% reduction of DPPH. All determinations were assayed in triplicate (Brand-Williams et al., 1995, Akter et al., 2010, Ismail et al., 2017).

2.7.2. ABTS Free Radical Scavenging Activity Assay

The free radical scavenging capacity of *F. zagrica* and *T. kurdica* plants bulb extracts were also measured using TEAC (Trolox equivalent antioxidant capacity) method. The TEAC value is based on the ability of the antioxidant to scavenge the blue-green coloured $ABTS^{\bullet+}$ radical cation relative to the $ABTS^{\bullet+}$ radical cation scavenging ability of the water-soluble vitamin E analogue, Trolox (Re et al., 1999, Gliszczyńska-Świgło, 2006). ABTS stock solution was dissolved in water to

a 7 mM concentration. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and the mixture was allowed to stand in dark at room temperature for 12-16 h. Prior to use, ABTS radical cation was diluted in methanol until the absorbance at 0.7 ± 0.02 units. ABTS solution (300 µl) was added to 30 µl sample and incubated for 6 min at room temperature. The absorbance was read at 734 nm. Ascorbic acid was chosen as positive control. The radical-scavenging activity was expressed as inhibition percentage, and calculated using the following formula.

$$\text{ABTS Radical scavenging activity} = \frac{Ac - As}{Ac} \; x \; 100$$

where *Ac* is the absorbance of reaction without samples and *As* is the absorbance of tested extract. The antioxidant activity was as median effective concentration (EC50) where the concentration caused 50% reduction of ABTS (Gliszczynskaswiglo, 2006, Ismail et al., 2017).

2.8. Statistical Analysis

Results of all analysis are presented as means of triplicate ± standard deviation (SD) and/or standard error (n = 3). Median effective concentrations (EC50) were statistically analyzed by GraphPad Prism 6 software. Non-linear regressions of log(agonist) vs. response with variable slope (four parameters) was selected for EC50 and LogEC50 estimation. The bottom and top constrain were 0% and 100% respectively. Additionally, statistical analysis was performed using GraphPad Prism 6 software with two-way ANOVA and Tukey's multiple comparisons post hoc test and Dunnett's multiple comparisons test. The results were considered statistically significant at P value < 0.05. Furthermore, Pearson correlation coefficient (r) was used find the relationships between the total phenolic and flavonoid contents and various antioxidant assays. The P value < 0.05 was considered significant.

3. RESULTS AND DISCUSSION

3.1. Extraction Yield

Extracts of bulbs of both *F. zagrica* and *T. kurdica* were obtained following a sequential solvent extraction procedure. Different organic solvents of increasing polarity including ethanol, methanol, and water were used to determine if any of these solvents could selectively extract compounds with antioxidant and antimicrobial activities. The yield percentages of *F. zagrica* and *T. kurdica* bulb extracts were expressed in terms of mass percentages of samples as mentioned in Table 1 and Figure 3. Extraction yield was calculated using following formula:

Extraction yield (%) = (weight of the freeze-dried extract x 100) / (weight of the original sample).

Results showed that the methanol bulb extracts of both *F. zagrica* and *T. kurdica* provide highest yields of extracts when compared to 80 % ethanol and aqueous extracts. The higher yield of methanol extract might be owed to the fact that methanol possesses high vapor pressure. Although the solubility of bio-active components and the rate of mass transfer are different, the results validated that nature and polarity of solvent affect the percentage yield of the extract. This was in agreement with the study by Marvibaigi, et al. (2016), who reported a high yield of mistletoe (*Scurrula ferruginea*) extracts when using polar solvents (Marvibaigi et al., 2016). The lowest percentage yield was exhibited for bulb aqueous extract of *F. zagrica*, while the highest yield was observed in the bulb

methanol extract of *F. zagrica*. In addition to the lowest percentage yield was recorded for bulb aqueous extract of *T. kurdica*, while the highest yield was revealed in the bulb methanol extract of *T. kurdica*. Large variations were showed between extraction yields of methanol, 80 % ethanol and aqueous extracts for both medicinal plants. Also using 80% water-ethanol solvent observed higher yield than using water alone to bulb extracts of both *F. zagrica* and *T. kurdica* plants. The results of other studies showed that the highest extraction yields with aqueous solutions might be ascribed to an increase of polarity of the solvents by adding water having a high dielectric constant (Kim et al., 2004, Kallel et al., 2014), which corroborate results of the present study. Hence, the sequence for increasing extraction yields were methanol extract > 80% ethanol extract > water extract.

3.2. Assay of Antimicrobial Activity against Pathogenic Organisms

This is the first report on the antibacterial screening and antifungal activity of *F. zagrica* and *T. kurdica* growing from Kurdistan Region-Iraq. Plants and their secondary metabolites have shown great potential as antibacterial and antifungal source. The first step towards this goal is the *in-vitro* antimicrobial activity. The antibacterial and antifungal assay of bulb extracts of *F. zagrica* and *T. kurdica* were performed against some pathogenic bacterial and fungal strains. The results in Table 2 and Figure 4 elucidated that *F. zagrica* and *T. kurdica* bulb extractions by using different solvents had strong antibacterial activities against all using bacterial strains (Gram positive and Gram negative bacteria) according to positive standard control (amikacin).

Maximum zones of inhibition of *F. zagrica* aqueous extracts (15.0, 26.33, 25.33 mm) were noticed against each of *E. coli ATCC 25922, A. baumannii ATCC 19606* and *S. aureus ATCC 25923,* respectively. While maximum zones of inhibition of *P. aeruginosa ATCC 27853* (24.0 mm) and *B. subtilis ATCC 6633* (27.67 mm) were found for each of *F. zagrica* methanol extract and *F. zagrica* ethanol extracts, respectively.

Tulipa kurdica aqueous extracts were showed maximum zones of inhibition (25.0, 31.0, 26.67 mm) against each of *E. coli ATCC 25922, P. aeruginosa ATCC 27853* and *S. aureus ATCC 25923* respectively, whereas ethanol extracts of *T. kurdica* were exhibited maximum inhibition zones against *A. baumannii ATCC 19606* (27.0 mm) and *B. subtilis ATCC 6633* (30.0 mm). The difference in antibacterial activity of different bulb extracts may be due to difference in solubility of bioactive compounds in various solvents and variations in cell wall structure of tested bacteria. The mode of action of different bioactive compounds varies which represent the ability of these compounds to kill or inhibit bacteria (Howell, 2007).

Aqueous extract of *F. zagrica* and *T. kurdica* showed better antibacterial activity compared to other solvent extracts. The findings of the present study are in agreement with previous investigation, which concluded that water extract exhibited higher antibacterial activity than ethanol extract of leaves and bark of *Cassia alata*, when tested against *C. albicans* and *S. aureus* (Somchit et al., 2003, Chen et al., 2018). The broad antibacterial activities of the Garlic (*Allium sativum* L.) and *Sida rhombifolia* Linn extracts can probably be attributed to the presence of various bio-actives components such as the phenolic (Kallel et al., 2014) alkaloid and flavonoid compounds (Dzoyem et al., 2010). As previously mention in the present that polar solvents are frequently

worked for the recovery of polyphenols from a plant matrix.

Fritillaria zagrica and *T. kurdica* methanol, 80% ethanol and aqueous extracts like many other plants showed antibacterial activity. Kumaraswamy *et al.* (2008) revealed that methanol, ethanol and aqueous *Betula utilis* D extracts had significant activity against some human pathogenic bacteria. Also Moon *et al.* (2011) showed that methanol and ethanol bulb extracts from *F. unibracteata* dose-dependently have inhibitory effect and increased antimicrobial activity, the strongest inhibition showed at the highest concentration of 20 mg/mL (Moon et al., 2011). Johnson and his colleagues (2011) screened five important medicinal plants, and the results observed that the maximum of Aloevera plant was to be exposed against *S. aureus* and *E. coli*, while *Lanatacamara* inactive against bacterial strains (Johnson et al., 2011). However, the aqueous fraction of the *Pongamia pinnata* had more active as compared to alcoholic extract against *E. coli*.

In other side Shigetomi et al. (2013) demonstrated that the natural products 1-tuliposide B and the lactonized aglycon (±)-tulipaline B in bulb *Tulipa* are potent inhibitors of MurA, which may partly explain the known antibacterial activity of these compounds (Shigetomi et al., 2013) and recently, a potent antibacterial activity of 6-tuliposide B has been reported (Shigetomi et al., 2010) . Also Tuliposides in bulb *Tulipa* had been reported to reveal antimicrobial activities and the formation of tulipalins played a key role in antimicrobial action (Shigetomi et al., 2011, Lim, 2014).

The results Table 3 and Figure 5 demonstrated the antifungal activities of *F. zagrica* and *T. kurdica* bulb extractions by using different solvents. *F. zagrica* methanol extracts were observed the maximum inhibition zones (18.0, 15.0, 9.0 mm) against *C. tropicalis ATCC 13803, C. albicans ATCC 10231* and *C. guilliermondii ATCC 6260*, respectively.

The *T. kurdica* bulb (methanol, 80% ethanol and aqueous) extracts showed antifungal activities against *C. tropicalis ATCC 13803* with the maximum inhibition zone (16 mm) of 80% ethanol extract. The *T. kurdica* 80% ethanol and aqueous, extracts were exhibited antifungal activities against *C. albicans ATCC 10231*. While The *T. kurdica* bulb (methanol, 80% ethanol and aqueous) extracts had no antifungal effect against *C. guilliermondii ATCC 6260.*

In parallel to our finding, previous studies were showed that *T. kurdica* bulb extracts have potent Tuliposides and tulipalins compounds, which exhibited their antifungal activity (Shigetomi et al., 2011). Bergman et al. (1967) isolated and identified α- methylene- butyrolactone (tulipan A), a fungitoxic substance from tulips bulb. Accordant with Moon et al. (2011) who were concluded that methanol and ethanol bulb extracts of *F. unibracteata* have potent anti-microbial activity (for example *C. albicans*) for the supportive treatment on respiratory diseases, food poisoning, and gastroenteritis diseases.

3.3. Total Phenolic and Flavonoid Content

Table 4 and 5 summarize the TPC and TFC in methanol, 80% ethanol and water bulb extracts of both *F. zagrica* and *T. kurdica* medicinal plants. The TPC values varied widely, ranging from 21.83 mg GAE/100 g samples to 153.7 mg GAE/100 g samples. MF exhibited the highest phenolic content at 153.7 mg GAE/100 g sample followed by EF (150.4 mg GAE/100 g sample), AF (139.4 mg GAE/100 g sample), MT (32.61 mg GAE/100 g sample), AT (29.16 mg GAE/100 g sample)

and ET (21.83 mg GAE/100 g sample). In another hand, the TFC detected varied from 80.12 CE/100 g sample to 365.5 mg CE/100 g sample. The highest flavonoid content was in MF at 365.5 mg CE/100 g sample followed by EF (179.2 mg CE/100 g sample), MT (130.3 mg CE/100 g sample), AF (114.4 mg CE/100 g sample) ET (99.49 mg CE/100 g sample) and AT (80.12 mg CE/100 g sample). TPC and TFC of *F. zagrica* and *T. kurdica* were statistically different from each other ($P < 0.05$), these two phytochemical compounds in methanol, 80% ethanol and aqueous extracts of both plants were statistically different from each other ($P < 0.05$) as shown in Figure 6 and 7.

Furthermore, results of the current study represented that different plant extracts contained different levels of TPC and TFC. *F. zagrica* contain significantly higher phenolic content than *T. kurdica*. The total phenol and flavonoid contents of the extracts were very near to those most medicinal plants. Results of the current study were in agreement with the previous studies (Marvibaigi et al., 2016), which presented that methanolic extracts of *Scurrula ferruginea* were rich in phenolic and flavonoid compounds. The presence of large amounts of phenolic compounds in the methanol, 80% ethanol and aqueous extracts may contribute to the antioxidant activities and the ability to adsorb and scavenge free radicals (Kumar et al., 2014).

3.4. DPPH and ABTS Free Radicals Scavenging Activities and Their Correlation to Phenolic and Flavonoid Contents

DPPH is a stable free radical, when antioxidant reacts with $DPPH^{\bullet}$ the electron is paired off and the DPPH solution is decolorized and $DPPH^{\bullet}$ provides an easy and rapid way to evaluate the antiradical activities of antioxidants. For that reason, the antioxidant activities were determined using DPPH as a free radical (Brand-Williams et al., 1995). The medicinal plants showing strong scavenging capacity on DPPH•, which is possibly due to the hydrogen donating ability of the polyphenolic (phenolic and flavonoid) compounds in the extracts (Mazhar, 2014, Bhagat et al., 2011).

The antioxidant activities of the *F. zagrica* and *T. kurdica* bulb extracts were assessed by different *in vitro* tests; DPPH and ABTS radical scavenging activities as presented in Figure 8. The EC50 calculated for DPPH (Table 6) were (2.006 2.661 µg/ml, 2.961 µg/ml, 9.241 µg/ml, 11.88 µg/ml and 21.77 µg ml^{-1}) µg/ml for MF, EF, AF, ET, MT and AT respectively. Meanwhile, a similar trend was mentioned (Table 6) in ABTS in which the EC50 for EF, AF, MT, MF, ET, and AT were (0.1535, 0.1611, 0.4435, 1.147, 1.532 and 2.493 µg ml^{-1}) respectively. Scavenging of DPPH radicals is mechanized by the donation of hydrogen atom to the unpaired electron of nitrogen bridge causing the purple color turn to yellowish. Meanwhile, the $ABTS^{+}$ radical cation undergoes the reduction process by hydrogen donating antioxidant and can be spectrophotometrically measured. It is that the phenolic and flavonoid content significantly influence the antioxidant activities (Othman et al., 2014, Ismail et al., 2017).

This original method quantified scavenging capacity by measuring a test compound at different concentrations and calculating the compound concentration required to reduce the initial DPPH concentration by 50% at steady-state (EC50). A lower EC50 value is associated with a stronger DPPH and ABTS radical scavenging capacity under the same testing conditions. Higher antioxidant activity is related to lower EC50 value (Liangli, 2008). Based on DPPH

and ABTS radical scavenging activity analysis, all bulb extracts of *F. zagrica* and *T. kurdica* represented antioxidant activity. The lowest EC50 value of DPPH was gained from methanol bulb extracts (MF) followed by ethanol (EF) and aqueous (AF) bulb extracts of *F. zagrica* while the lowest EC50 value was obtained from ethanol (ET) bulb extracts followed by methanol (MT) and aqueous (AT) bulb extracts of *T. kurdica*. The lowest EC50 value of DPPH in methanol bulb extract may due to the fact that methanol is better solvent than the others in extracting phenolic compounds from the extracts due to their polarity and good solubility for phenolic components from different plant materials such as walnut green husk, cacao bean husk and wild rice hulls (Kallel et al., 2014). Thus the samples with higher total phenols content showed the higher antioxidant properties (lower EC50 values). The results of present study indicated that increased concentrations resulted in enhancing the scavenging capacity of bulb extract samples. Broad variation of antioxidant activity may be possibly attributable to the presence of wide range of biologically active components like phenols, flavonols, carotenoids and some other compounds (Marvibaigi et al., 2014). Additionally, Liu et al (2012) indicated that a water-soluble polysaccharide (FUP-1) was obtained from *Fritillaria ussuriensis* Maxim, exerts antioxidant activity not only through its own radical-scavenging activity but also by boosting the enzymatic and non-enzymatic antioxidant defense system of the host.

From the observation, the extracted compound from the 70% ethanol and methanol gave higher antioxidant activity compared to the absolute solvent and Turkmen et al. (2006) reported the same findings where they found 50% and 80% of solvent mixture exhibited considerably higher DPPH radicals scavenging activity compared to the pure solvent (Turkmen *et al.*, 2006).

Besides, the (aqueous) bulb extract illustrated low antioxidant potential (higher EC50 values of DPPH and ABTS) and also low content of total phenols as compared to the other bulb extracts produced with methanol and 80 % ethanol solvents. Similar results have been reported during the extraction of antioxidant compounds from other raw materials such as mango peel and seed (Dorta et al., 2012), grape by-products (Lapornik et al., 2005).

In the present study Table 7 represents the correlation coefficients of the possible correlation between the phenolic and flavonoid contents of *F. zagrica* and *T. kurdica* bulb extracts and their antioxidant activities (DPPH and ABTS). It also observes the correlation between different methods used. The TPC and TFC showed a significant and positive linear correlation ($p<0.05$) with different antioxidant activity assays (DPPH and ABTS activities). These results suggested that the antioxidant activity is more closely related to TPC than TFC. Our findings exhibited a strong (higher) positive correlation between TPC and antioxidant activity assays (R^2 = 0.8261 and 0.5861 of DPPH and ABTS, respectively) and also proved that the phenolic compounds were the major contributors to the antioxidant capacity of the *F. zagrica* and *T. kurdica* bulb extracts. Similarly, Turkmen et al. (2006) who declared that the results of black mate tea showed that solvent with different polarity had significant effect on polyphenol content and antioxidant activity and a high correlation between polyphenol content and antioxidant activity of tea extracts was observed (Turkmen et al., 2006).

Moreover, Pearson correlation analysis of the results revealed a significant and positive correlation between different antioxidant

assays ($p<0.05$). The highest correlation was elucidated between ABTS and DPPH ($r = 0.7601$), whereas ABTS that could scavenge $ABTS^{\bullet+}$ was also able to scavenge $DPPH^{\bullet}$. Furthermore, the strong correlation between DPPH and ABTS methods suggests that the antioxidants in the extracts react similarly with both assays. The findings of the present study are in agreement with previous investigation of Ismail et al. (2017) who reported that radical scavenging of DPPH and ABTS were positively correlated to the phenolic and flavonoid content of tested herbal plants, which include *Andrographis paniculata* (leaves), *Cinnamon zeylanicum* (bark), *Curcuma xanthorrhiza* (rhizome), *Eugenia polyantha* (leaves) and *Orthosiphon stamineus* (whole plant). Based on our knowledge there is no report on TPC and TFC and antioxidant activities of *F. zagrica* and *T. kurdica* bulb extracts.

4. CONCLUSIONS

The present study demonstrated and proven that *F. zagrica* and *T. kurdica* bulb extracts in different solvents were exhibited strong antibacterial activities against all tested bacterial strains (Gram positive and Gram negative bacteria), and fungal strains except *C. guilliermondii ATCC 6260*. Aqueous bulb extract of *F. zagrica* and *T. kurdica* showed highest antibacterial activity compared to other solvent extracts.

Additionally, the phenolic and flavonoid content were varied between the tested bulb extracts and the DPPH and ABTS scavenging activities were found to be significantly correlated with the amount of TPC and TFC. Moreover, methanol *F. zagrica* bulb extracts contain significantly higher phenolic content than *T. kurdica*. Also demonstrated that the phenolic compounds were the major contributors to the antioxidant capacity of the *F. zagrica* and *T. kurdica* bulb extracts.

The strong antioxidant abilities of *F. zagrica* and *T. kurdica* along with their traditional use in the treatment of various ailments be suggesting their power potential as natural antioxidants and providing the scientific rationale to obtain pure compounds and then develop new therapeutic drugs against breast cancer with accomplishing cytotoxicity and anticancer screening for further detailed analysis in the future.

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Conflict of Interest (1)

Table (1) Percentage yield of two medicinal bulb extracts in different solvents

	Yields (%)		
Bulb Extract	**Methanol**	**80%Ethanol**	**Aqueous**
F. zagrica	7.76 ± 0.18*	2.97 ± 0.15	2.60 ± 0.08
T. kurdica	7.40 ± 1.1*	4.70 ± 0.18	3.57 ± 0.2

All results are means of three replicates determinations ± SD (n = 3).

***** Represents significant difference at $p < 0.05$.

Table (2) Antibacterial screening test [zone of inhibition (mm)] of *F. zagrica* and *T. kurdica* bulb extracts

(10mg/mL) in different solvents against some bacterial pathogenic strains.

Bulb Extract	Inhibition zones (mm)				
	Gram (-ve) pathogenic bacteria			*Gram (+ve) pathogenic bacteria*	
	E. coli ATCC 25922	***P. aeruginosa ATCC 27853***	***A. baumannii ATCC 19606***	***S. aureus ATCC 25923***	***B. subtilis ATCC 6633***
MF	12.33 ± 0.33[a]	24.00 ± 0.58	19.00 ± 1.16[a]	19.33 ± 0.33	24.67 ± 0.33[a]
EF	12.33 ± 0.67[a]	13.00 ± 0.58	20.67 ± 0.67[a]	15.33 ± 0.88[a]	27.67 ± 1.45[a]
AF	15.00 ± 0.58[a]	19.67 ± 0.88[a]	26.33 ± 0.88	25.33 ± 0.88	25.00 ± 0.58[a]
MT	20.00 ± 1.16	10.00 ± 1.16	15.00 ± 0.58[a]	15.33 ± 0.88[a]	25.00 ± 0.58[a]
ET	22.00 ± 1.16	25.67 ± 0.67	27.00 ±1.00	21.67 ± 0.88	30.00 ± 0.58
AT	25.00 ± 0.58	31.00 ± 1.00	23.67 ± 0.88	26.67 ± 1.20	28.67 ± 0.33
PC	14.67 ± 0.33	19.67 ± 0.88	18.00 ± 0.58	14.00 ± 0.58	24.67 ± 0.88

Data are means of three replicates (n = 3) ± standard error.

[a] Not statistically different from positive control (PC).

Table (3) Antifungal activities [zone of inhibition (mm)] of *F. zagrica* and *T. kurdica* bulb extracts against some pathogenic fungal organisms (1 mg/mL).

Fungal Pathogenic Organism	*F. zagrica*			*T. kurdica*		
	MF	**EF**	**AF**	**MT**	**ET**	**AT**
C. tropicalis ATCC 13803	18	14	15	12	16	11
C. albicans ATCC 10231	15	14	13	0	10	11
C. guilliermondii ATCC 6260	9	8	6	0	0	0

Data analysis by Two-way ANOVA (p<0.05). There are significant differences among *Candida* spp. (p value = 0.0002) and among bulb extracts of both medicinal plants (p value = 0.0153).

(0): No Antifungal inhibitory activity.

Table (4) Total phenolic content of two medicinal bulb extracts in different solvents

Bulb Extract	TPC (mg GAE/100 g sample)		
	Methanol	**80%Ethanol**	**Aqueous**
F. zagrica	153.7 ± 1.415^{ax*}	150.4 ± 0.65^{bx}	139.4 ± 0.50^{cx}
T. kurdica	32.61 ± 0.18^{ay}	21.83 ± 0.53^{by}	29.16 ± 0.44^{cy}

All results are means of three replicates determinations ± SD (n = 3).

* Represents significant difference at $p < 0.05$.

Table (5) Total Flavonoid content of two medicinal bulb extracts in different solvents

TFC (CE/100 g of sample)			
Bulb Extract	**Methanol**	**80% Ethanol**	**Aqueous**
F. zagrica	365.5 ± 20.01	179.2 ± 12.11	114.4 ± 2.48
T. kurdica	130.3 ± 1.24	99.49 ±13.46	80.12 ± 1.42

All results are means of three replicates determinations ± SD (n = 3).

***** Represents significant difference at $p < 0.05$.

Table (6) Mean of TPC, TFC and EC50 of DPPH and ABTS radical scavenging activity.

Bulb Extract	Total phenolic content (mg GAE/100g sample)	Total flavonoid content (mg CE/100g sample)	EC50 - DPPH RSA (µg/mL)	EC50 – ABTS RSA (µg/mL)
MF	153.7 ± 1.415	365.5 ± 20.01	2.006	1.147
MT	32.61 ± 0.18	130.3 ± 1.24	11.88	0.4435
EF	150.4 ± 0.65	179.2 ± 12.11	2.661	0.1535
ET	21.83 ± 0.53	99.49 ±13.46	9.241	1.532
AF	139.4 ± 0.50	114.4 ± 2.48	2.961	0.1611

AT	29.16 0.44	±	80.12 1.42	±	21.77	2.493

Each value in the table is represented as mean (n = 3).

Table (7) Pearson correlation coefficients between antioxidant activities and TPC/ TFC of *F. zagrica* and *T. kurdica* bulb extracts (at significance P < 0.05).

Assays	**Correlation coefficients (R^2)**			
	TPC	**TFC**	**DPPH**	**ABTS**
TPC	1.0000	0.6528*	0.8261**	0.5861*
TFC	0.6528*	1.0000	0.5923*	0.1697
DPPH	0.8261**	0.5923*	1.0000	0.7601*
ABTS	0.5861*	0.1697	0.7601*	1.0000

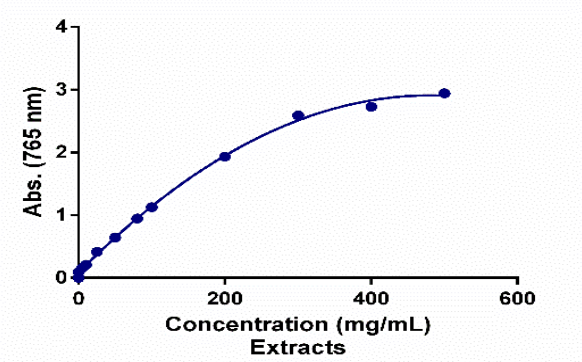

Figure 1: Standard curve graph of Total phenolic content (TPC).

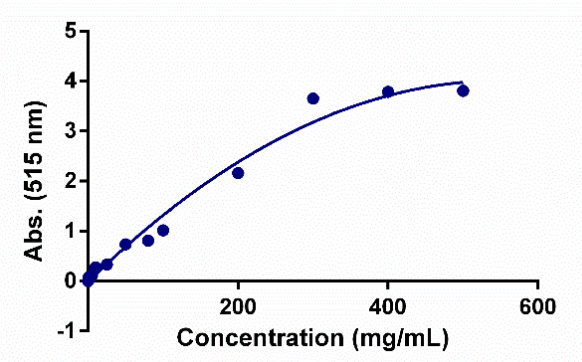

Figure 2: Standard curve graph of Total Flavonoid Content (TFC).

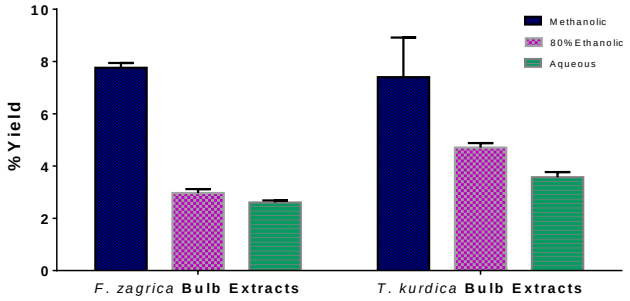

Figure 3: Percentage of *F. zagrica* and *T. kurdica* bulb extraction yields obtained by using different solvents.

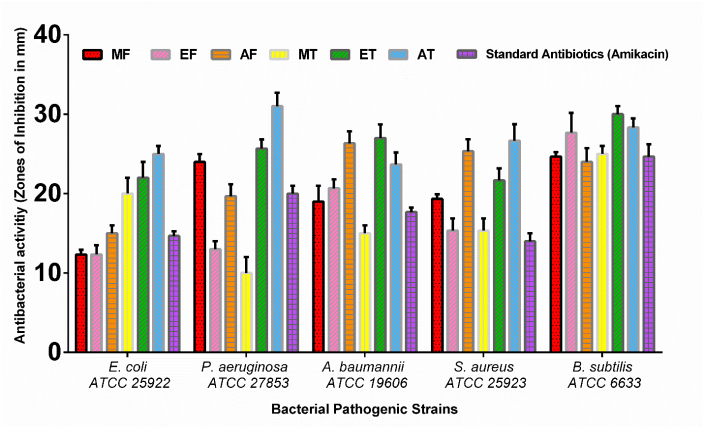

Figure 4: Antibacterial screening test of *F. zagrica* and *T. kurdica* bulb extracts in different solvents against some bacterial pathogenic strains.

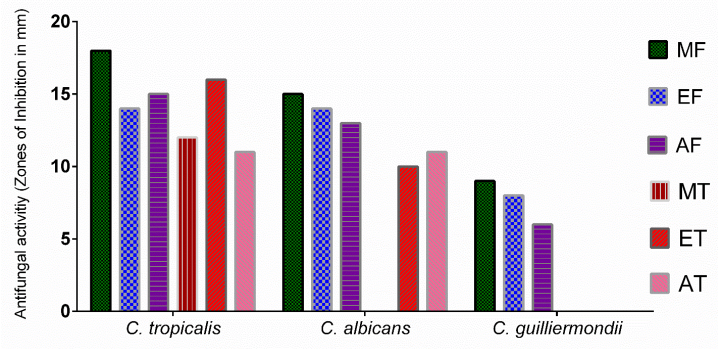

Figure 5: Antifungal activities of *F. zagrica* and *T. kurdica* bulb extracts against some pathogenic fungal organisms (1 mg/mL).

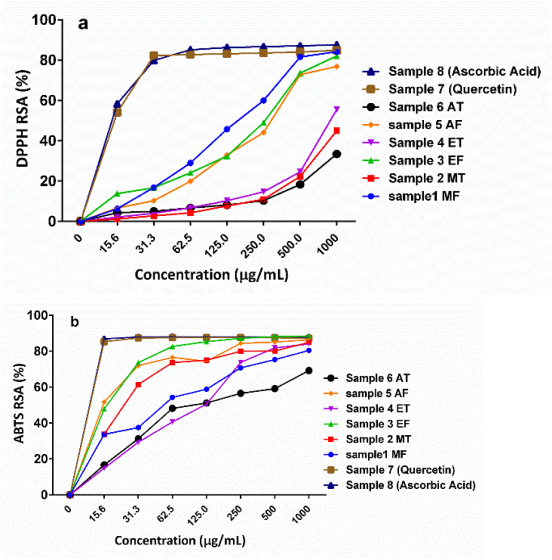

Figure 8: Antioxidant activities of MF, MT, EF, ET, AF and AT of *F. zagrica* and *T. kurdica* at various concentrations ranging from 15.60 to 1000 mg/ml. a) DPPH• radical scavenging activities. b) ABTS radical scavenging activities. Ascorbic acid and quercetin were used as standard (Positive controls). Results were expressed in means ± SEM (n = 3).

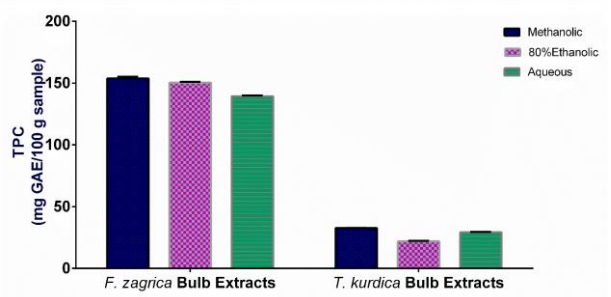

Figure 6: Total phenolic content (TPC) of *F. zagrica* and *T. kurdica* bulb extractions obtained by using different solvents.

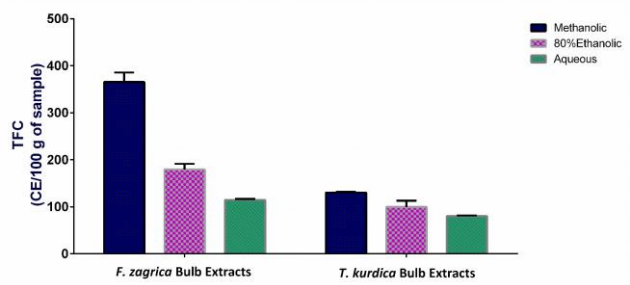

Figure 7: Total flavonoid content of *F. zagrica* and *T. kurdica* bulb extractions obtained by using different solvents.

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