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Original article

Antibacterial and antioxidant potential of some Egyptian medicinal plants used in traditional medicine

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ABSTRACT

Objectives: Medicinal plants continue to gain popularity on a global scale. Besides, there is a need for discovering new antibacterial natural extracts that could be used as antibiotics alternatives against resistant bacteria. In this respect, the aim of this study was to determine the phytochemicals, antioxidant activity, calorific nutritional value and antibacterial potential of four traditionally used wild medicinal plants (*Achillea fragrantissima (Delile) Hayne, Teucrium polium L., Peganum harmala L.* and *Solenostemma argel (Forssk.) Sch. Bip.* grow in Saint Catherine Protectorate, South Sinai, Egypt.

Methods: Standard methods were applied to determine the proximate composition, calorific nutritional value, secondary metabolites (phenolics and flavonoids), antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$), ferrous ion chelating (FIC), 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid ($ABTS^{\cdot+}$) and Ferric reducing antioxidant power (FRAP) techniques in addition to the antibacterial potential of the active aqueous extracts by broth dilution technique.

Results: The results obtained illustrated that *T. polium* recorded the highest nutritional calorific value (205.63 ± 5.76 calories/100 g dried plant), total phenolics (8.057 ± 0.322 g gallic acid equivalent/100 g dried plant), total flavonoids (2.013 ± 0.034 g catechin equivalent/ 100 g dried plant and antioxidant scavenging activity using DPPH (EC_{50} = 1.84 mg extract/g DPPH), FIC (IC_{50} = 0.068 mg extract/ml), ABTS (61.11%) and FRAP (2185.71 mmol Fe (II)/g extract) assays followed descendingly by *A. fragrantissima, S. argel* and *P. harmala,* respectively. There was direct relation between the flavonoids/ phenolics ratios and the radical scavenging activity among all extracts. Regarding the antibacterial potential, the extracts expressed broader antibacterial spectrum against *Bacillus cereus* (ATCC®11778™), *Bacillus subtilis* (ATCC®19659™), *Escherichia coli* (ATCC®10536™), *klebsiella pneumoniae* (ATCC®10031™), *Listeria innocua* (ATCC®33090™), *Listeria monocytogenes* (ATCC®19115™), *Pseudomonas aeruginosa* (ATCC®9027™), *salmonella enterica* (ATCC®15479™) and *Salmonella typhimurium*)ATCC®14028™(strains. The minimal inhibitory concentrations of the extracts estimated using broth dilution assay ranged from 0.049 to 1.56 mg/ml. *T. polium* extract possessed the highest activity among all other extracts.

Conclusions: In conclusion, the studied medicinal plants could be used as nutritional supplements, antioxidants and antibacterial botanicals for combating some of the pathogenic bacteria.

Abbreviations: ABTS, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; AE, antiradical efficiency; Cfu, colony forming unit; CAZ, ceftazidime antibiotic; DPPH, 2,2-diphenyl −1- picrylhydrazyl; FIC, the ferrous ion chelating; FRAP, ferric reducing antioxidant power; MIC, minimal inhibitory concentration; NCCLS, the national committee for clinical laboratory standards; SAM, ampicillin-Sulbactam; TPTZ, 2, 4, 6 – tripyridyl - s - triazine.

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1. Introduction

The Egyptian flora comprises about 2185 species that represent an important source of medically important compounds (Boulos, 2005). González-Tejero et al., (2008) concluded that the wild plant species, including the target species selected for the present study, play an important role in Bedouin's daily life and that their work in the Mediterranean region construct a base for consequent phytochemical and pharmacological researches that could lead to new therapeutic products. Traditionally, the herbal extracts in form of tea decoction of these species are used by local Bedouins in remote areas for the treatment of many illnesses like diabetes, allergy, kidney and respiratory and digestive problems (Baydoun et al., 2015).

The environmental stresses in the natural habitats of wild plants enhance them to synthesize active ingredients with high therapeutic potential like phenolics and flavonoids that possess distinguished antioxidant activity and therapeutic effects that contributes to heart diseases, neurodegenerative diseases, cancer and aging process (El-Zayat et al., 2021; Kurutas, 2016; Tungmunnithum et al., 2018; Zargoosh et al., 2019).

The increased incidence of bacterial resistance to antibiotics, has forced the research towards alternatives, like traditionally used medicinal plants and combinational therapies (Cheesman et al., 2017; El-Shahaby et al., 2019; Thomford et al., 2018).

Therefore, based on local community surveys, this research aimed to evaluate the nutritional value, phytochemicals, antioxidant activity and antibacterial potential of four Egyptian medicinal plants used in folklore medicine at Saint Catherine Protectorate.

2. Materials and methods

2.1. Plants collection

The aerial parts of *Solenostemma argel, Teucrium polium*, *Achillea fragrantissima* and *Peganum harmala* at vegetative stage were gathered from Saint Catherine Protectorate, South Sinai, Egypt in the period from October to November 2018. They were taxonomically identified according to Boulos (1999–2002). The plants were air dried for 15 days, grinded and kept in dry containers (Table 1).

2.2. Extracts preparation

Aqueous extracts were prepared in the same manner of administration of plants in folklore medicine as tea decoction. 10 g of the dried plants were extracted in 100 ml distilled water using shaking water-bath at 65 °C for 30 min then evaporated until dryness using rotary evaporator.

2.3. Phytochemical analysis

2.3.1. Primary metabolic variables

The metabolic variables including fats, proteins and total carbohydrates content were estimated in the investigated plants on dry weight moisture free basis. Fats were determined according to Arlington (1995), proteins according to Bradford protein assay (Bradford, 1976) and the total carbohydrates according to Thayumanavan and Sadasivam, (1984).

The nutritional value was calculated according to the formula:

Nutritional calorific value = 4.1x proteins % + 9.2x fats % + 4.1x total carbohydrates %.

The energy produced expressed as calories /100 g dried plant (AOAC, 2016).

2.3.2. Secondary active compounds

2.3.2.1. The total phenolics were quantified using Folin Ciocalteu technique adopted by Wolfe et al (2003) and estimated as gm gallic acid equivalent / 100 gm dried plant concerning the standard curve (y = 0.0064x, r2 = 0.99).

2.3.2.2. The total flavonoids were quantified by $AlCl_3$ colorimetric technique adopted by Zhishen et al (1999) and expressed as gm catechin equivalent /100 gm dried plant concerning the standard curve (y = 0.004 ×, r2 = 0.99).

2.3.3. Antioxidant scavenging activity

2.3.3.1.2,2- diphenyl –1- picrylhydrazyl (DPPH) assay was used for determination of the antioxidant scavenging activity as adopted by Kitts et al. (2000) and modified by Liyana Pathirana and Shahidi (2005). 1 ml of the aqueous extracts with different concentrations was introduced to 1 ml of 0.135 mM DPPH•. The absorbance was detected at 520 nm after 30 min at exclusion of light. The remaining $DPPH^{\cdot}$ % was calculated using the formula:

$$\%\text{DPPH inhibition} = \left[DPPH^0 \right]_T / \left[DPPH^0 \right]_{T=0} X\ 100$$

and graphed against mg extract/ gm $DPPH^{\cdot}$ using exponential curve to estimate EC_{50} and against mg extract/ ml to estimate IC_{50} (The concentration of the extract capable of diminishing 50% of the initial DPPH concentration) (Sanchez Moreno et al., 1998). EC_{50} is inversely proportional to the antioxidant efficacy (AE = 1/ EC_{50}) (Spranger et al., 2008). Gallic acid and ascorbic acid were employed as standards.

2.3.3.1.2,2- diphenyl –1- picrylhydrazyl (DPPH) assay was adopted by Singh and Rajini (2004). 1 ml of 2 mM ferrous sulphate was added to 1 ml of the extracts and 1 ml 5 mM ferrozine then incubated for 10 min. The absorbance was estimaed at 562 nm. The concentration at which 50% of iron(II) chelated (IC_{50}) was

Table 1
Ethnobotanical information of the studied plants (González-Tejero et al., 2008; Boulos, 1999–2002).

Botanical name	Family	Local name	Habitat	Part used	Traditional use	Mode of use
Solenostemma argel (Del)	Asclepiadaceae	Hargal	Sand plains & terraces	Air dried aerial parts	Cough, colds, gastro-intestinal cramps, stomachic, anti-colic, urinary tract and liver problems, syphilis	Tea decoction
Achillea fragrantissima (Forssk) Sch. Bip.	Asteraceae	Gysoume	Wadi beds & gorges		Stomach ache and stomach worms eye pain, and infected wounds.	Tea decoction cold infusion
Teucrium polium (L.)	Lamiaceae	Jaada	Rocks & cliffs, gorges and wadi beds		Allergy, stomach, colic pains and fattiness	Tea decoction
Peganum harmala (L.)	Zygophyllaceae	Harmal	Sand plains		Leaves and flowers are used as anti rheumatic, teeth pain & stomach problems.	Tea decoction

calculated where it is inversely proportional to the antioxidant power. Gallic acid and ascorbic acid were employed as standards.

2.3.3.3 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid ($ABTS^{+}$) method adopted by Re et al. (1999) to measure the antioxidant scavenging activity of the extracts. $ABTS^{+}$ cation radical was released on basis of the reaction between 7 mM $ABTS^{+}$ and 2.45 mM $K_2S_2O_8$ (1:1), kept in dark at room temperature for 16 h then diluted with methanol until reaching absorbance of 0.700 at 734 nm. 5 µl of each extract (contain 1.05 mg) was added to 3.995 ml $ABTS^{+}$ solution and left for 30 min. The absorbance inhibition percent was measured at 734 nm using the equation:

$$ABTS^{+}\text{scavenging}(\%) = ((AB\text{--}AA)/AB) \times 100$$

Where, AB is the absorbance of $ABTS^{+}$ and methanol; AA is the absorbance of $ABTS^{+}$ and extract.

Ascorbic acid and gallic acids were employed as standards.

2.3.3.4.Ferric reducing antioxidant power (FRAP) method was adopted by Benzie and Strain (1996) to determine the antioxidant potential of the extracts. This method aimed to reduce Fe3 + - TPTZ complex to Fe^{2+}-tripyridyltriazine by the electron donating antioxidants. 300 mM acetate buffer with pH 3.6 were introduced to 10 mM TPTZ (2,4,6 – tripyridyl -s- triazine) and 20 mM FeCl3. 6 H2O in ratio of 10:1:1 to prepare FRAP solution. $FeSO_4$ $7H_2O$ was used as Standard. 3.6 ml of FRAP solution were introduced to 0.395 ml water and left at 37° C for 5 min then mixed with 5 µl of the extract (contain 1.05 mg) and left for 10 min at 37° C. The absorbance was measured at 593 nm.

2.4. Determination of the antibacterial potential

2.4.1. Agar well diffusion technique

The antibacterial potential of the aqueous extracts was determined by the agar well diffusion technique according to the national committee for clinical laboratory standards (NCCLS, 1993; Valgas et al., 2007). The antibiotics, Ceftazidime and Ampicillin-Sulbactam were employed as positive controls.

2.4.2. Broth dilution technique

Stocks of the aqueous plant extracts were used to produce different dilutions in the range of 0.006 to 6.250 mg/ml. Each dilution was seeded with bacterial culture (10^6 cfu/ml) and incubated for 24 h at 37° C. The bacterial growth was observed as turbidity and the least concentration at which no turbidity was observed is the minimal inhibitory concentration (MIC) (NCCLS, 1993; Wiegand et al., 2008).

2.4.3. Bacterial strains

Bacillus cereus (ATCC®11778™), *Bacillus subtilis* (ATCC®19659™), *Enterobacter cloacae* (ATCC®23355™), *Escherichia coli* (ATCC®10536™), *Klebsiella pneumonia* (ATCC®10031™), *Listeria innocua* (ATCC®33090™), *Listeria monocytogenes* (ATCC®19115™), *Proteus vulgaris* (ATCC®49132™), *Pseudomonas aeruginosa* (ATCC®9027™), *Salmonella enterica* (ATCC®15479™), *Salmonella typhimurium*) ATCC®14028™ (, *Staphylococcus aureus* (ATCC®6538™), *Staphylococcus epidermidis* (ATCC®12228™) and *Streptococcus pyogenes* (ATCC®19615™).

The tested microorganisms were of animal origin and obtained from MERCIN center at Ain Shams University.

3. Results

3.1. Metabolic variables

The proximate composition of the investigated plants is presented in Fig. 1. *T. polium* recorded the maximum content of crude fats and total carbohydrates, while *S. argel* recorded the maximum of proteins. In the mean time, *P. harmala* recorded the minimum content of crude fats and total carbohydrates. *A. fragrantissima* attained the minimum value of proteins among the investigated species. *T. polium* and *S. arghel* expressed the highest nutritive value followed by *A. fragrantissima* while that of *P. harmala* showed the least nutritive value as presented in Fig. 2.

3.2. Total phenolics and flavonoids

The values of phenolics varied from 3.34 ± 0.094 to 8.06 ± 0.322 gm gallic acid equivalent/100 gm dried plant while the flavonoids

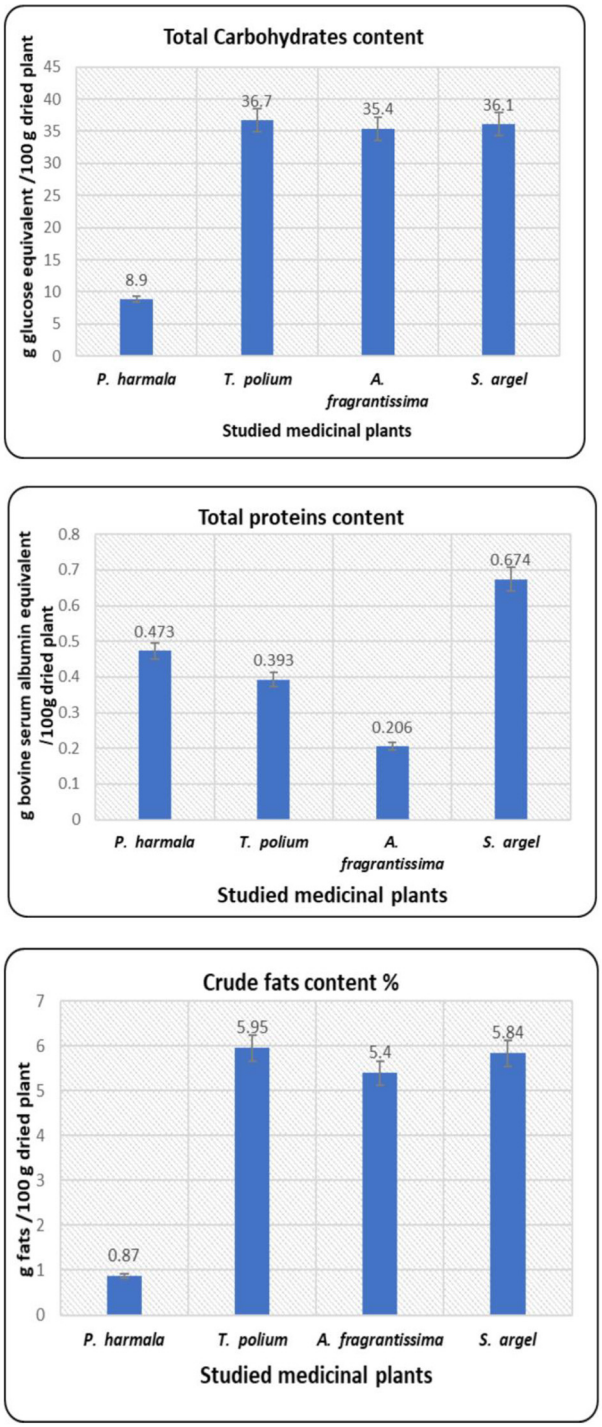

Fig. 1. The estimated metabolic variables in the studied species.

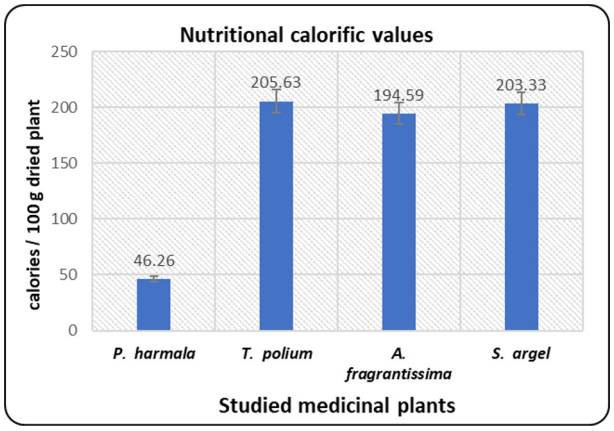

Fig. 2. The nutritional calorific values of the studied plants.

varied from 0.134 ± 0.004 to 2.013 ± 0.034 gm catechin equivalent/100 gm dried plant.

The highest phenolics content was in *T. polium*, followed by *P. harmala, A. fragrantissima and S. argel*, respectively. *T. polium* attained the highest flavonoids content, followed by *A. fragrantissima*, *S. argel* and *P. harmala*, respectively (Fig. 3).

3.3. Evaluation of the antioxidant activity

The antioxidant scavenging activity of the extracts was determined using different assays as presented in (Table 2).

DPPH and FIC assays illustrated that *T. polium* extract expressed the highest antioxidant scavenging activity followed by *A. fragrantissima* while *S. argel* showed moderate activity and *Peganum harmala* found to be the lowest.

ABTS$^{\cdot+}$ radical cation decolorization assay showed that the aqueous extract of *T. polium* was the most active as it nearly scavenged 81.11% ABTS$^{\cdot+}$ followed by that of *A. fragrantissima* (73.54%), *S. argel* (23.77%) and *P. harmala* (7.81%), respectively.

Regarding the FRAP assay the antioxidant potential of each extract was evaluated on the basis of their ability to the reduction of TPTZ-Fe^{3+} complex to TPTZ-Fe^{2+} complex. The antioxidant potential of *T. polium* (2185.71 mmol Fe (II)/g extract) was higher than that of *A. fragrantissima, S. argel* and *P. harmala,* respectively.

The results revealed that the total flavonoids/ total phenolics ratio is directly proportional to the free radical scavenging activity of the infusions (Fig. 4).

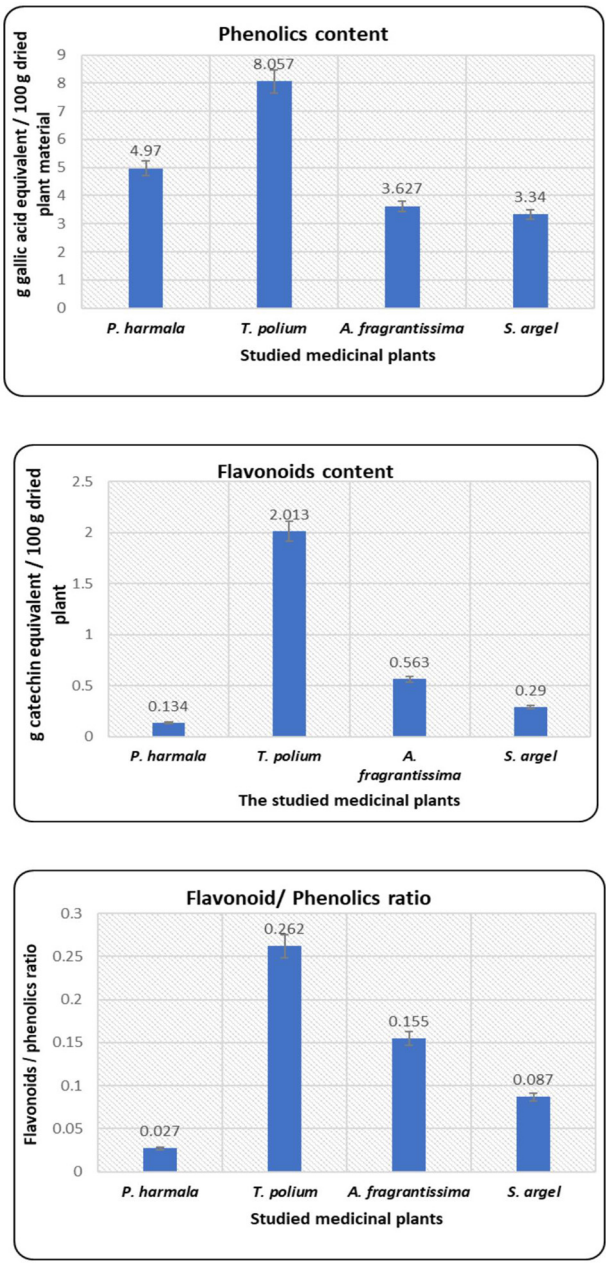

Fig. 3. Total phenolics, flavonoids and flavonoids/ phenolics ratio in the extracts.

3.4. Antibacterial activity

T. polium exhibited the broadest antimicrobial spectrum against 64.28% of the tested pathogens followed by *S. argel* (57.24%), *A. fragrantissma* (50%) while the extract of *P. harmal* exhibited the least antimicrobial spectrum (42.86%) (Fig. 5). None of the extracts exhibited activity against *S. aureus, S. epidermidis, E. cloacae, P. vulgaris* and *S. pyogenes* (Table 3).

The MIC values < 100 μg/ml have been proposed to be highly active, 100–500 μg/ml active, 500–1000 μg/ml moderately active and 1000–2000 μg/ml less active (Silva et al., 2013). Accordingly,

Table 2
The antioxidant scavenging activity of the extracts.

Plant Species	DPPH			FIC	ABTS	FRAP
	IC_{50} (mg extract/ml)	EC_{50} (mg extract/gm DPPH)	AE	IC_{50} (mg extract/ml	% of Inhibition	mmol Fe (II)/gm extract
S. argel	1.20	35.25	0.028	1.27	23.77%	112.5
A. fragrantissima	0.15	4.13	0.242	0.149	73.54%	972.32
T. polium	0.07	1.84	0.544	0.068	81.11%	2185.71
P. harmala	4.70	130.73	0.008	4.706	7.81%	32.14
Ascorbic acid	0.02	0.610	1.64	0.022	88.86%	6589.29
Gallic acid	0.02	0.525	1.91	0.019	90.18%	7664.11

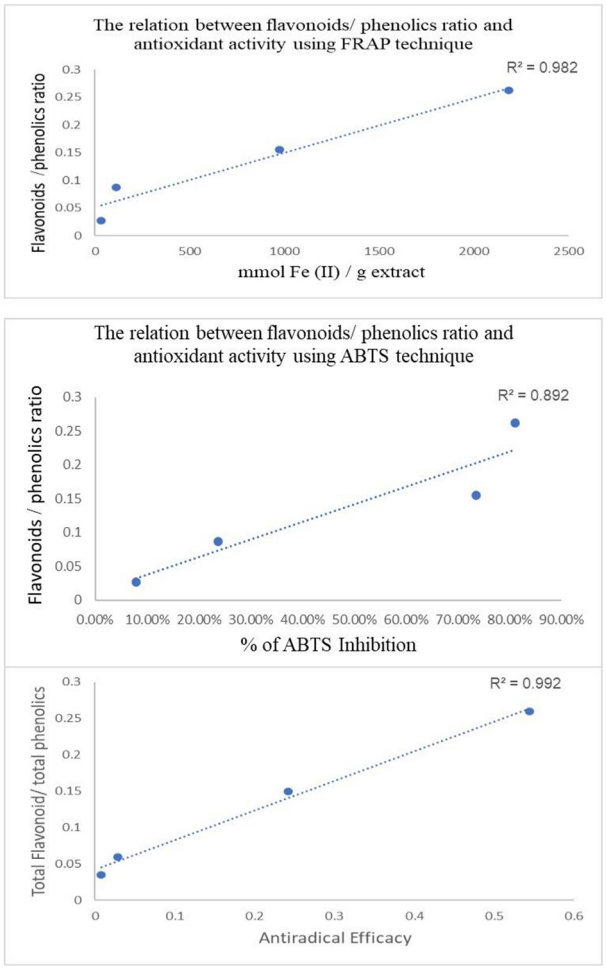

Fig. 4. The relation between flavonoids/ phenolics ratio and antioxidant activity.

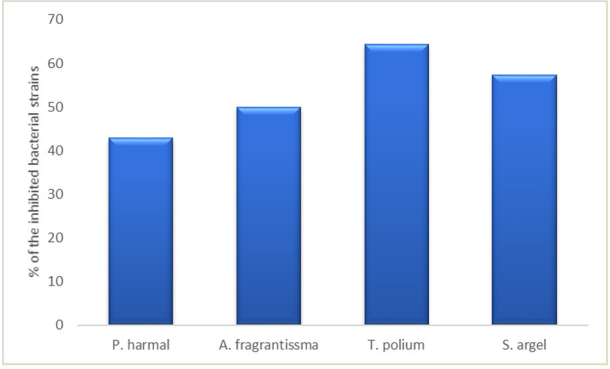

Fig. 5. % of the inhibited bacterial strains by each of the tested extracts.

T. polium extract was highly active against *S. enterica, B. subtilis, B. cereus* and *L. monocytogenes*, active against *E. coli, L. innocua* and *P. aeruginosa* and moderately active against *K. pneumoniae. A. fragrantissma* was highly active against *S. enterica* and *B. subtilis* and active against *E. coli, K. pneumoniae, B. cereus, L. innocua* and *P. aeruginosa. S. argel* extract was highly active against *S. typhimurium* and *B. subtilis,* active against *S. enterica* and *E. coli,* moderately active against *P. aeruginosa* and *L. innocua* and with very low activity against *B. cereus. P. harmal* extract was active against *K. pneumoniae* and *S. enterica* as presented in Table 4.

Table 3
Antibacterial potential of *the water infusions of the studied plants.*

Pathogenic bacteria	Inhibition zones in millimeters				Standard Antibiotic	
	P. harmal	*A. fragrantissma*	*T. polium*	*S. argel*	SAM	CAZ
Gram negative bacteria						
S. typhimurium	10	–	15	20	15	20
P. aeruginosa	14	14	19	15	R	19
E. cloacae	–	–	–	–	10	20
K. pneumoniae	15	16	17	15	15	28
P. vulgaris	–	–	–	–	12	25
E. coli	12	14	23	14	15	24
S. enterica	20	20	28	17	R	28
Gram positive bacteria						
S. epidermidis	–	–	–	–	15	8
B. subtilis	–	18	25	20	20	24
B. cereus	–	15	20	12	13	7
S. aureus	–	–	–	–	12	20
S. pyogenes	–	–	–	–	R	25
L. innocua	11	13	17	14	17	18
L. monocytogenes	–	–	19	–	34	13

*(CAZ): Ceftazidime, (SAM)**:** Ampicillin/Sulbactam, R: Resistance, the diameter of the well (8.0 mm) is included in the measured zone of inhibition

Table 4
Minimal inhibitory concentrations of the extracts estimated by broth dilution assay.

Plant species	*P. harmal*	*A. fragrantissma*	*T. polium*	*S. argel*
Bacterial strains	MIC (**mg sample/ml)**			
Gram negative bacteria				
S. typhimurium	1.56	–	0.781	0.097
P. aeruginosa	1.56	0.390	0.390	0.781
K. pneumoniae	0.195	0.195	0.781	1.56
E. coli	1.56	0.390	0.195	0.390
S. enterica	0.390	0.049	0.097	0.195
Gram positive bacteria				
B. subtilis	–	0.097	0.049	0.097
B. cereus	–	0.195	0.097	3.125
L. innocua	1.56	0.195	0.195	0.781
L. monocytogenes	–	–	0.097	–

4. Discussion

Herbal medicines and their use in treating and preventing certain diseases worldwide and in Egypt have increased potentially (Shaito et al., 2020). The primary metabolites estimated in the studied plants are essentially required due to their nutritional calorific value. The obtained results illustrated that *T. polium, S. argel* and *A. fragrantissima* contain appreciable levels of nutritive content within appropriate limits of using in feeding stuffs. Primary metabolites are essential for plant growth and development, besides their role in determining the nutritional quality (Sagwan et al, 2010; Tungmunnithum et al., 2018; Zargoosh et al., 2019).

T. polium expressed the highest antioxidant activity, phenolics and flavonoids content, followed by *A. fragrantissima*, *S. argel* and *P. harmal,* respectively. The therapeutic benefits of medicinal plants are often related to their antioxidant properties that attributed to the phenolics and flavonoids content in the extracts (Esmaeili et al., 2015). It was also clear from the results that, flavonoids/ phenolics ratio is directly proportional to the antioxidant activity of the extracts. Khazaei et al. (2018) reported that *T. polium* possesses antioxidant and free radical scavenging activity in addition to its effect against peroxidation.

The misuse of antibiotics in addition to the lack of developing new medications has forced the search for antimicrobials of natural origin (Othman et al, 2019). Medicinal plants possess potent pharmacological properties, lower toxicity, and economic

potentiality due to the presence of phenolics, flavonoids, tannins and alkaloids (Shakya, 2016; Atef et al., 2019). Phytoactive components solely or in combination with antibiotics may be considered effective antimicrobials (Gupta and Birdi, 2017). The results proved variable antibacterial spectrum of the studied extracts with remarkable MIC values as *T. polium* exhibited the broadest spectrum followed by *S. argel, A. fragrantissima* and *P. harmal*, respectively. Accordingly, the activities observed in this study are. The results agree with that reported by Khazaei et al. (2018) that *T. polium* aqueous extract possesses broad antibacterial potential.

5. Conclusion

In conclusion, the studied medicinal plants could be used as source of nutritional supplements, antioxidants, drugs and antibacterial botanicals for combating of the studied pathogenic bacteria. The studied extracts could be used as food preservatives and as antibacterial that protect animals and human from pathogenic bacterial diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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