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Biological properties and identification of some active ingredients in *Anastatica hierochuntica* and *Lepidium sativum,* grown in Egypt

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Abstract

Medicinal plants have been used as a source of therapies since ancient times in Egypt. The present study was designed to investigate and compare between the anti-oxidant, anti-bacterial, and anti-tumor activity of different extracts from *Anastatica hierochuntica* and *Lepidium sativum* belong to Brassicaceae, cultivated in Egypt. The qualitative phytochemical screening followed by DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay were used to assess the anti-oxidant of the extracts, while the disk diffusion method followed by micro-broth dilution were used to determine minimum inhibitory concentration of the plant extracts against 6 bacterial strains belonging to 3 species, *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus,* The all results show that the studied plants have various bioactive phytochemicals. Both plants were observed to be moderate antioxidant agents but *L. sativum* was better than *A. hierochuntica*. Moreover, all studied crude extracts were able to inhibit the growth of reference and clinical isolated bacteria while *L. sativum* was more effective. A human ovarian adenocarcinoma OV17R, cell line was used to evaluate antitumor of these extracts by Sulfo-Rhodamine B colorimetric (SRB) assay and IC_{50} values below 20 µg/mL were recorded for the crude extract of both tested plants. Benzoic acid, cinnamic acid, rutin and vanillin were the most abundant phenolic compounds in extracts. Rutin and benzoic acid showed the best anti-bacterial activity against all tested bacteria, also expressed moderate cytotoxic activity with IC_{50} 28.8 and 37.2 µg/mL, respectively. The present investigation provided supportive data for the possible use of the plant extracts investigated here in treatment of various diseases.

Keywords: Medicinal plants; Anti-bacterial; Anti-tumor; Phytochemicals; DPPH; HPLC

1. Introduction

From ancient times, medicinal plants are important in developed and developing countries and act as a source of primary health care that has been used by people all over the world in the form of folk medicines. According to the World Health Organization, 25% of modern drugs used in America have been derived from medicinal plants [1]. With evolving scientific advancement, the unique properties of these natural products have been studied and analyzed for chemical and structural diversity in relation to their therapeutic and other biological activities. Further, the medicinal plant species growing in different environments are known to have variations in their chemical constituents. Accordingly, it is important to analyze these medicinal plants as well as it is essential to preserve and maintain wild indigenous plants that are of economic and medicinal use. The ancient Egyptians were aware with many medicinal herbs in treatment of different diseases. They used plant organs such as leaves, seeds, and oils and applied it in the form of powders, creams, and ointments however, scientific evidence for their medicinal properties are not proved [2, 3]

Brassicaceae is one of the largest angiosperm families. The plants of this family are used in the treatment of many diseases because of their anticancer, antibacterial, antifungal, anti-rheumatic and anti-diabetic properties [4].

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Anastatica hierochuntica (known as blooming virgin hand, locally called Kaf Mariam) and *Lepidium sativum* (known as garden cress, locally called Hub Alrachad) are small, annual herbs and cultivated in different regions of Egypt. Apart from Egypt , these are found in arid areas of other Arab countries such as Qatar, Saudi Arabia, Oman, United Arab Emirates, Kuwait and Iraq, as well as in some South Asian, African and European countries.

The preparations of *A. hierochuntica* is used to overcome menstrual cramps, prevent uterine hemorrhage in pregnant women and facilitate smooth delivery [5]. The commonly found phytochemical constituents with biological activities identified in various parts (leaves, stem and seeds) of this plant—using ethanol, methanol, ethyl acetate, hexane and aqueous extracts—include tannins, sterols, terpenes, flavonoids, alkaloids, saponins, resins, phenols and glycosides [6]. These phytochemicals (natural products or secondary metabolites) act as a natural defense system for host plants and provide color, aroma and flavor. Herbal tea preparations from seeds of *A. hierochuntica* (commonly consumed in Saudi Arabia) are known to exhibit antioxidant activity [7]. The antioxidant is defined as any substance that, when present in low concentration compared with those of an oxidizable substrate, it prevents or repairs the process of oxidation of that substrate. It is well known that oxidant by-products of normal metabolism such as free radicals and reactive oxygen species (ROS) in excess leading to various degenerative diseases of aging such as arthritis, cancer, cardiovascular disease, immune system decline, and brain dysfunction [8]. Many synthetic antioxidants are used currently, but these substances are inappropriate for chronic human consumption, as recent publications have stated their toxic properties for human health. Hence, the development of alternative antioxidants from plant origin has attracted significant attention and is believed to be a required development [9]**.** The compounds predominantly present in the tea preparations of *A. hierochuntica* include phenolic acids, chlorogenic acids and flavonoids [7]. It is also important to note that the polar and non-polar phenolic compounds present in *A. hierochuntica* have been recently attributed to the nephroprotective, antioxidant and free radical scavenging activities [10]. The aqueous, ethanol and methanol extracts of *A. hierochuntica* have been shown to exhibit antibacterial activity against number of pathogenic bacteria which can lead to serious and life threatening complications such as sepsis, kidney and liver failure, toxic shock, and even death if it untreated [11].

L. sativum is one of the mucilage containing fast growing, edible annual herb, the obovate pods contain two seeds and are around 5 mm in length. A plant's volatile oils can be found in its seeds and leaves. Natives of Egypt, Saudi Arabia, Sudan, and other Arabic countries know all about the benefits of the *L. sativum* plant and its seeds for facilitating the healing of bone fractures. The free radical scavenging activity of total phenolic and flavonoid compounds extracted from seeds has been reported and showed maximum antioxidant activity by inhibiting DPPH and hydroxyl radical, super oxide anion scavenging, nitric oxide and hydrogen peroxide scavenging activities than the reference standards studied [12]. The effectiveness of *L. sativum*'s extracts as antibiotics against some pathogens like *S. aureus, E. coli, P. aeruginosa,* and *C. albicans* was investigated and the results were promising. Due to side effects of synthetic medicine, medicinal plants are gaining regard over these drugs because of their lesser side effects and low resistance against microorganisms [13]. The fact that natural active compounds have a significant advantage to provide new commercial drugs and considered a hopeful starting point. Accordingly the aim of this study is to investigate and compare the anti-oxidant, antibacterial, and antitumor activity of the aqueous and organic extracts of *A. hierochuntica* and *L. sativum* grown in Egypt.

2. Material and methods

2.1. Plant samples and extraction

A. hierochuntica (Branches, seeds, bods) and *L. sativum* (seeds) were harvested from their natural habitats in the villages of Sheikh Zuweid Center near South Rafah in North Sinai, Egypt and identified in Agricultural Research Center. The fresh samples were dried at 50 ℃, then reduced to fine particles using Waring laboratory blender (MX-7011G) for 5 min at high speed and then stored in airtight closed bottles for two days before being used for analysis. For aqueous extracts, 300 gm dried ground plant material was soaked in 1L distilled water and heated in water bath at 40 ℃ for 5 hours For organic extract, 300 gm plant material was percolated with 1L of organic solvent (ethanol and aceton) in glass bottles, these bottles were vigorously shaken at a speed of 300 rpm, overnight. All filtrates obtained were concentrated under reduced pressure (at 68 ℃) in a rotary evaporator to obtain the crude extract which kept at 4 ℃ until further uses. The percentage yield of extract for different solvents was calculated using the formula: Weight of final extract/ Weight of powdered X100.

2.2. Qualitative phytochemical analysis

Preliminary Phytochemical analysis for Flavonoids, Alkaloids, Glycosids, Terpenes, Phenolics, Saponins, Tannins were carried out using standard protocol as described by Ashfaq et al. [14].

2.3. DPPH assay

The antioxidant activity of plant material was assayed by DPPH assay. 10 µl of plant extract (1mg/mL) was added to 100 µL of 0.2 mM DPPH solution in a microtiter plate. The reaction mixture was incubated at 25 ℃ for 5 min, then measured at 520 nm. The DPPH without plant material served as the control. The methanol with respective plant extracts serves as blank. The percent DPPH scavenging activity was calculated as: [(AB- AT) / AB] X 100, where AB and AT are the absorbance of blank and plant material, respectively [15].

2.4. Antibacterial activity

2.4.1. Microorganisms

The test organisms for in-vitro antibacterial screening were three reference microorganisms "*P. aeruginosa* (NCTC 10662), *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), and three clinical isolated MDR bacteria "*S. aureus*, *E. coli*, *P. aeruginosa*, which isolated and identified by automated biochemical tests using Vitek®MS colorimetric identification card (bioMerieux - Marcy-l'Étoile, France). The susceptibility patterns were obtained using Vitek®MS aspartate amino-transferase. One single colony of each tested microorganism, taken from nutrient agar stock cultures into 10 mL sterile Muller-Hinton broth medium then incubated at 37 ℃ for 16 - 20 hours.

2.4.2. Disc diffusion assay

10 µl of each plant extract (100 mg/mL) was applied to each filter paper disc to give a final amount of 1 mg plant extract per disc. The discs were air dried and placed on top of the agar layer and incubated for 16-20 hours at 37 ℃. Standard antibiotic discs served as positive control. All antimicrobial studies were done in triplicates [16].

2.4.3. Determination of MIC and MBC

Serial dilution of each extract that showed significant zones of inhibition were individually placed in tubes filled with 1 mL M-H broth including bacterial suspension. After 24 hours incubation at 37 ℃, turbidity was taken as an indication of growth, and the lowest concentrations which did not show any turbidity was determined as MIC. In order to determine MBC values, 100 µL of the content of the tubes with no turbidity were cultured on the M-H agar medium and incubated at 37 ℃ for 24 hours [17].

2.5. Potential Anticancer Assay

2.5.1. Cell Culture

OV17R, cell line (96020763, Sigma Ald.) was used, starting from a frozen ampoule, add thawed cells to 4 mL of RPMI 1640 medium (R8758, Sigma Ald.) in the tube. Centrifuge the cell suspension at low speed for 5 min. The media was removed and re-suspend the cell pellet at a density of 1 x 104 cells/mL in fresh medium supplemented with 10% FBS “fetal bovine serum” (v/v) and 100 mg/mL streptomycin and 100 IU/mL penicillin at 37 ℃ in CO_2 incubator with 5% CO_2.

2.5.2. SRB (Sulforhodamine B colorimetric) Assay

100 µL of cells (1×104 cells/mL) was seeded in 96-well plates and incubated at 37 ℃, 5% CO_2. After 24 hours of incubation, the cells were treated with 100 µL of different concentrations of plant extracts (0.0, 12.5, 25.0, 50.0 and 100.0) µg/mL. Plates were incubated at 37 ℃, 5% CO_2. After 48 hours incubation, cells were washed and stained with 20 µL of SRB stain. The plates were further incubated for 4 hours and excess stain was washed with 1% acetic acid, then attached stain was recovered by "Tris EDTA" buffer. The wells with only culture medium treated with 1% of DMSO served as control. The absorbance was measured at 560 nm for each well and the relation between surviving fraction and extract conc. was plotted to get the survival curve. Survival fraction (S.F.) = Absorbance sample / Absorbance control. Then the half maximal inhibitory concentration (IC_{50}) was calculated for each extract after 24 hours of exposure from survival curve [18].

2.6. HPLC for Phenolic Compounds in Plant Extract

2.6.1. Sample and Standard Compounds Preparation

Using ethanol HPLC spectral grade, 10 mg/mL of plant extracts and 10 µg/mL of sixteen different pure known phenolic compounds as external standards (gallic acid, catechol, p- hydroxy benzoic acid, caffeine, vanillic acid, caffeic acid, syringic acid, vanillin, ferulic acid, rutin, ellagic acid, benzoic acid, salicylic acid and cinnamic acid were prepared before injection in the analytical HPLC system and chromatographed.

2.6.2. HPLC System

Chromatographic analysis were carried out using Agilent 1260 Infinity HPLC Series (Agilent, USA), equipped with Quaternary Pump (G1311B), Autosampler (G1329B, ALS) and Diode Array Detector G1315D, VL) coupled to Agilent Open LAB ChemStation B.04.03 software. Phenolic compounds were separated on a ZORBAX Eclipse plus C18 reversed-phase column (100 mm x 4.6 mm, 5 µm) (Agilent technologies, USA).

2.6.3. Chromatographic Conditions

At 25 ℃, the separation is achieved using a ternary linear elution gradient with solvent A (HPLC grade water and 0.2% H3PO4 (v/v) at pH 2.65), solvent B (methanol) and solvent C (acetonitrile) with flow rate 0.7 mL/min. The injected volume of plant extract was 20 µL VWD detector set at 284 nm. The concentration of an individual compound was calculated on the basis of peak area measurements [19].

2.6.4. HPLC Analysis and Identification of Compounds

Based on a combination of retention time (Rt) and spectral matching with those of pure standard, the results were expressed as area % of each identified compound from the total area.

2.7. Screening of Antibacterial and Cytotoxicity of Some Identified Phytochemicals

Based on the results obtained by HPLC assay, some phenolic compounds, which were present in high concentration, were selected for evaluation of their antibacterial and antitumor activities. The stock solution of selected compounds in concentration of 50 mg/mL were prepared and the antibacterial activity, MIC, MBC and antitumor activity were applied according to the methods mentioned before.

2.8. Statistical analysis

Six or more replicates were obtained for each treatment and the data are expressed as standard error of the mean. Comparisons between groups were performed by using paired students t-test on a Statistical Software Package (SPSS). Differences were considered significant, if p value is less than 0.05 “$p < 0.05$”.

3. Results

The average percentage of extraction yields with ethanol was better than acetone and distilled water as solvents (Fig 1). Phytochemical screening provide the presence of flavonoids, phenolics, saponins, etc. in the organic extacts of the tested plants, however, water extract of them did not extract the flavenodis, alkaloids or phenolic compounds as shown in Table 1. All extracts of experimental plants produced moderate DPPH scavenging activity (< 90 - 40% Inhibition). (Fig 2).

Table 1 Qualitative chemical analysis of phytoconstituents in different extracts of tested plants

Tested plants	Flavonoids			Alkaloids			Glycosids			Terpenes			Phenolics			Carbohydrates			Proteins			Saponins			Tannins		
	a	b	C	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	C
A. hierochuntica	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L. sativum	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+

aq, hot water extract; eth, ethanol extract; ace, acetone extract, + : present; - :absent.

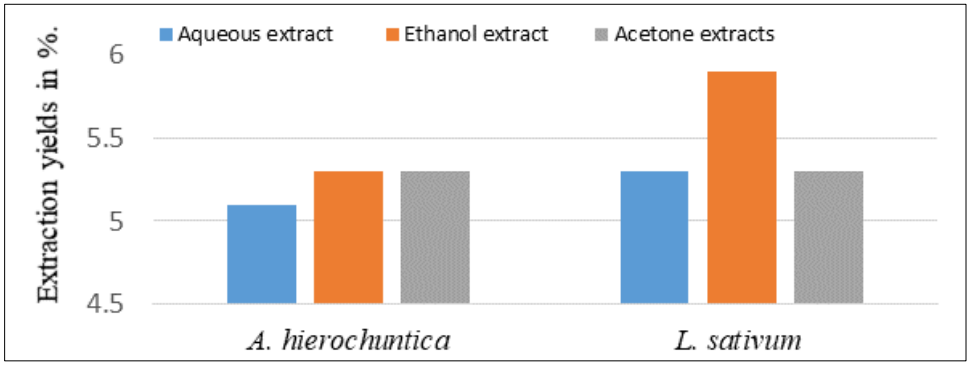

Figure 1 Selected medicinal plants and its extraction yields in %

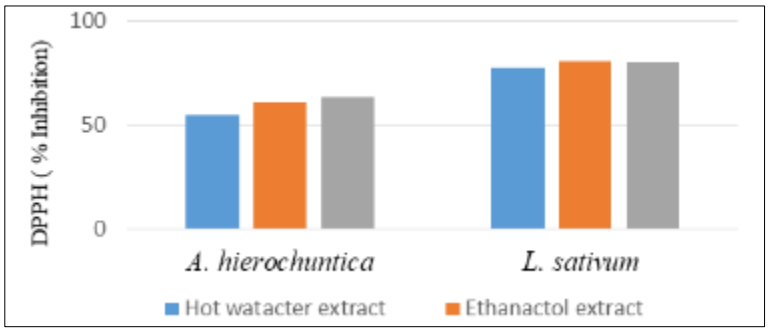

Figure 2 DPPH free radical scavenging activity (% Inhibition) of different extracts of tested medicinal plants

Table 2 The antibacterial activities (diameter of inhibition zone, mm) of *A. hierochuntica* and *L. sativum* tested bacteria

		***P. aeruginosa* NCTC 10662**	***E. coli* ATCC 25922**	***S. aureus* ATCC 25923**	**MβL, *P. aeruginosa***	**ESβL, *E. coli***	**MRSA**
A. hierochuntica	a	-	-	9.17 ± 0.24	-	-	-
	b	7.00 ± 0.41	9.33 ± 0.47	9.00 ± 0.00	7.17 ± 0.24	9.17 ± 0.24	7.00 ± 0.00
	c	9.67 ± 0.62	-	10.00 ± 0.82	-	7.17 ± 0.24	7.67 ± 0.62
L. sativum	a	7.17 ± 0.24	7.00 ± 0.00	7.33 ± 0.24	-	-	8.33 ± 0.47
	b	9.17 ± 0.24	15.50 ± 0.41	7.67 ± 0.47	7.17 ± 0.24	8.17 ± 0.62	8.00 ± 0.41
	c	7.00 ± 0.00	7.83 ± 0.62	8.33 ± 0.24	7.00 ± 0.00	7.17 ± 0.24	-
AMP		12.0 ± 0.41	15.50 ± 0.41	25.67 ± 0.47	20.33 ± 0.47	-	-
OFX		24.00 ± 0.00	29.50 ± 0.41	24.33 ± 0.47	16.83 ± 0.62	19.33 ± 0.47	20.33 ± 0.47
VA		-	-	22.50 ± 0.41	-	-	21.67 ± 0.47
CTX		22.17 ± 0.24	22.50 ± 0.41	18.50 ± 0.41	-	-	14.33 ± 0.47

- : No inhibition zone; aq: hot water extrac; eth: ethanol extract; ace: acetone extract; ESβL *E. coli*: Extended Spectrun β Lactamase producing *E. coli*; MβL *P. aeruginosa*: Metallo-beta-lactamase producing *P. aeruginosa*; MRSA: Methicillin-resistant *S. aureus*; AMP: Ampicillin 10 µg; OFX: Ofloxacine 10 µg; VA: Vancomycin 30 µg; CTX: Cefotaxime 30 µg.

The results of antimicrobial action showed that *L. sativum* was stronger antibacterial activity compared to *A. hierochuntica*. In addition, the result of inhibition zone confirmed that the ethanol extract was more effective than

aqueous and acetone extracts. (Table 2). MIC ranged from 3.13 to 200 mg/mL, the least was 3.13 mg/mL for *L. sativum* against *E. coli* ATCC 25922 and the highest was 200 mg/mL for *A. hierochuntica* against MRSA. The MBC ranged from 3.13 to 200 mg/mL, the least was 3.13 mg/mL for *L. sativum* against *E. coli* ATCC 25922 and the highest was 200 mg/mL for *A. hierochuntica* against*, S.* MβL*, P. aeruginosa* and MRSA (Table 3).

Table 3 MIC and MBC (mg/mL) of tested plant extracts against tested organisms

Plant extracts		***P. aeruginosa* NCTC 10662**	***E. coli* ATCC 25922**	***S. aureus* ATCC 25923**	**MβL, *P. aeruginosa***	**ESβL, *E. coli***	**MRSA**
A. hierochuntica	a	-	-	100/100	-	-	-
	b	100/100	100/100	50/50	100/200	50/100	200/200
	c	100/100	-	50/100	-	100/100	100/100
L. sativum	a	25/25	6.25/6.25	6.25/12.5	-	-	12.5/25
	b	12.5/25	3.13/3.13	6.25/6.25	25/25	12.5/12.5	12.5/25
	c	25/25	6.25/12.5	3.13/6.25	25/50	12.5/25	12.5/25

- : No antibacterial activity; aq: hot water extrac; eth: ethanol extract; ace: acetone extract; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration MDR: multi-drug resistant; ESβL *E. coli*: Extended Spectrun β Lactamase producing *E. coli*; MβL *P. aeruginosa*: Metallo-beta-lactamase producing *P. aeruginosa*; MRSA: Methicillin-resistant *S. aureus*.

L. sativum and *A. hierochuntica* were able to inhibit the proliferation of OV17R cell line (Fig 3) and induced more than 50% inhibition with IC_{50} values 15.7 and 16.6 µg/mL, respectively. The quantitative analysis of phenolic compounds expressed in mg/100 gm dry sample for *L. sativum* and *A. hierochuntica* by HPLC analysis was carried out, and data are tabulated in Table 4. P- Hydroxy benzoic acid, caffeic acid, vanillic acid, ferulic acid, sinapic acid, cinnamic acid, and rosmarinic acid, rutin, syringic acid were detected in both tested plants, while salicylic acid was absent in both. Caffeine, gallic acid, and cinnamic acid were detected in *L. sativum* in concentrations of 31.3, 5.3 and 18.4 mg/100 gm DW, respectively, while catechol, vanillic acid, and ellagic acid have been detected in *A. hierochuntica* (9.5, 2.05 and 12.95 mg/100 gm DW, respectively).

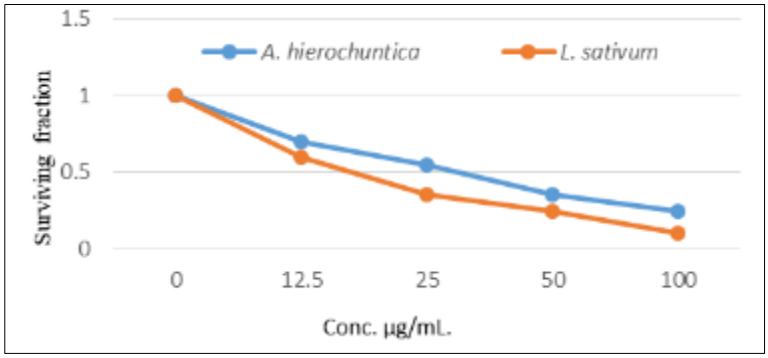

Figure 3 Potential antitumor assay of plant extracts at different conc. using OV17R, cell line

As can be seen, the predominant compounds within *A. hierochuntica* were sinapic acid, caffeic acid followed by rutin (65.42, 39.77, and 29.62 mg/100gm DW, respectively). These three substances together make up about 71% of total phenolic compounds that were identified in *A. hierochuntica*. Benzoic acid, rutin followed by vanillin were the major compounds in *L. sativum* (105.0, 98.87, and 53.8 mg/100 gm DW, respectively) and together make up about 70% of total identified phenolic compounds in *L. sativum*. Other detected phenolic compounds in both plant extracts were present in low to moderate concentrations. When comparing total content of the identified phenolic compounds in both *L. sativum* and *A. hierochuntica,* it was noticed that *L. sativum* contains about 90.9% phenolic compounds more than *A. hierochuntica.*

Table 4 The figure presents the conc. of identified free phenols (mg/100 gm DW) within *A. hierochuntica* and *L. sativum* using HPLC

Phenolic compound	***L. sativum***	***A. hierochuntica***
Gallic acid	5.3	ND
Catechol	ND	9.5
p- Hydroxy benzoic acid	21.3	1.87
Caffeine	31.3	ND
Vanillic acid	6.5	2.05
Caffeic acid	2.98	39.77
Syringic acid	3.4	7.6
Vanillin	53.80	8.48
Sinapic acid	11.10	1.84
Ferulic acid	9.20	2.85
Rutin	98.87	29.62
Ellagic acid	18.98	12.95
Benzoic acid	105.0	7.83
Rosmarinic acid	21.04	0.86
Salicylic acid	ND	ND
Cinnamic acid	18.4	65.42

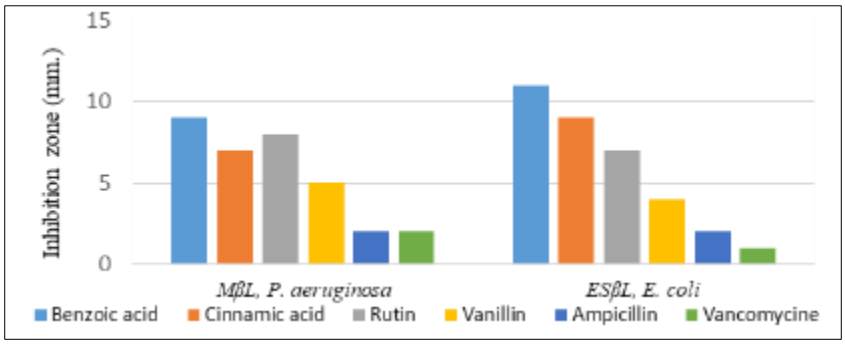

Figure 4 Antimicrobial assay of benzoic acid, cinnamic acid, rutin and vanillin against selected bacteria

In Fig 4, benzoic acid, cinnamic acid, rutin and vanillin showed antibacterial activity against all tested bacteria, better than ampicillin and vancomycine as reference antibiotics. Rutin produced greatest inhibition zone against *P. aeruginosa NCTC 10662* (20 mm), while benzoic acid showed the best pattern of inhibition against other tested bacteria. The results of antitumor assay of benzoic acid, cinnamic acid, rutin and vanillin in different concentrations (graphically represented in Fig 7) expressed a moderate antitumor activity of Rutin and Benzoic acid (IC^{50} 28.8 and 37.2 µg/mL, respectively), while cinnamic acid and vanillin showed weak antitumor activity (IC_{50} 59.8 and 64.32 µg/mL, respectively).

4. Discussion

Medicinal plants have a long therapeutic history and still considered to be promising source of medicine in the traditional health care system. Extraction is the main step for recovering and isolating phytochemicals from tested plants and its efficiency is affected by the extraction method and the solvent used, moreover, the percentage yields of the extraction depend on the polarity of solvents, temperature, pH, and extraction time [20]. Water, ethanol and acetone are commonly used for the extraction and the properties of extracting solvents effect on the total phenolic content and

anti-oxidant capacity [21]. The results in this study show that, the extraction yield increases with increasing polarity of the solvent used, this may be the reason why yields of ethanol, and acetone extracts are higher than yields of water. These results are in agreement with previous studies in which, extraction yields of rice bran and some medicinal plants decreased in the following order: ethanol > acetone > distilled water [22].

Baker *et al.* [23] reported the presence of tannins, flavanoids, glycosides, phenols, saponins and terpenes in different extracts of *A. hierochuntica*, and these results are in agreement with our results like Ahmed *et al.* [24] who stated presence of alkaloids, carbohydrates, flavonoids, saponins, phenols, and proteins in *L. sativum*.

DPPH method is a preferred method in antioxidant assay because it is fast, easy and does not require a special reaction. The results in this study are in agreement with Złotek *et al.* [25] who reported that the free radical scavenging potentials of both acetone and ethanol extracts are higher than hot water extracts. In a previous study, acetone was more effective solvent than methanol for phenols extraction, however, the best solvent for phenolic extraction from horseradish roots was ethanol. Moreover, the maximum polyphenols extraction was obtained in the alcoholic extract of *Bauhinia vahlii* followed by acetone, hot water and chloroform extracts [26]. Ihsanullah [27] reported that *A. hierochuntica* was rich in total phenolic content, and to have high free radical scavenging activities, while Indumathy and Aruna [28] evaluated the free radical scavenging activity of *L. sativum* by inhibiting DPPH and showed maximum activity. Recently, the antioxidant and free radical scavenging properties of natural products have generated tremendous research interest owing to their related anticancer properties, as well as their protective effects against cardiovascular diseases and their ability to confer decreased risk of certain chronic diseases, as reported by Ihsanullah [27]**.**

Bacterial infectious diseases represent an important cause of morbidity and mortality worldwide. Therefore, the development of new antibacterial agents for the treatment of bacterial infections is important. Our results indicated some plant extracts exhibited a good antibacterial activity and some others are limited as judged by their MIC values, these activities may be due to occurrence of different phytochemicals. The flavonoids and alkaloids have been found to possess anti-microbial and antioxidants properties in various studies [29]. The efficient anti-oxidant plant extracts showed remarkable anti-microbial activity, which may be refer to the poly-phenolic components and other phytochemicals which could be responsible for both activities. Our extracts showed the larger inhibition zone as well as low MIC values against tested bacteria [30]. It was noted that ethanol is a better solvents for more consistent extraction of antimicrobial substances from plants compared to water and acetone solvents [31]. Our results are supported by the study of Tomsone *et al.* [21] in which, he reported that the recovery of polyphenols from plant materials is influenced by the solubility of phenolic compounds in the solvent used.

In our results, all tested bacteria showed susceptibility toward ethanolic and acetone extracts of *L. sativum* and we observed that the ethanolic extract of *A. hierochuntica* showed antibacterial activities against tested bacteria [32]. Such observation was supported by Almundarij *et al.* [10] who found that ethanol extract of *A. hierochuntica* showed antibacterial effect against both Gram positive (*S. aureus*) and Gram negative bacteria (*E. coli*, *P. aeruginosa*). This discrepancy between the used plant extracts and the inhibiting degree of the tested strains can be explained by the fact that, the activity depends on the type, composition, and concentration of the plant extract, and the type of target micro-organisms. Many other factors could also be involved such as age of the plant used, freshness of plant, physical factors as temperature and light, time of harvesting, drying method, the effect of solvents and the extraction method on the phenolic contents, and seasonal or intraspecific variation of plant extract composition [33]. Moreover, the negative results do not mean that the bioactive constituents are absent or that the plant is inactive, but active compounds may be present in insufficient quantities so that, the doses level would not be enough to exhibit the inhibitory effect. It is also possible that the plant extracts may be active against other bacterial species that were not tested [32].

The results that have been obtained from antitumor activity showed strong activity according to plant screening program of the American National Cancer Institute US NCI which states that, a crude extract is generally considered to have *in vitro* strong antitumor activity if the IC_{50} value following incubation between 48 and 72 hours is less than 20 µg/mL [34]. Efati *et al.* [35] reported that *L. sativum* showed IC_{50} value of 17.8 µg/mL against SW480, HT-29 and Caco-2 colorectal cancer cell lines, also the cytotoxic activity of seed extracts of *L. sativum* was tested against human neuroblastoma (IMR-32), colon cancer (HT-15 and 29), and lung cancer (A-549) cell lines [28]. Al-Eisawi, *et al*, [36] observed the antitumor activity of all tested concentrations of *A. hierochuntica* against leukemia (K-562) and melanoma (A-375) cells. The antitumor activity of tested plants may be due to the presence of phytochemicals content in extracts. Even though there was increase in the cell growth inhibition when concentration of plant extract was increased.

HPLC is the best way for chemical characterization and determination of both composition and concentrations of the secondary metabolites of a sample [31]. Interestingly, current data confirmed that *A. hierochuntica* and *L. sativum* are

rich in phytochemicals compounds and is a good source of natural antioxidants with potential health benefits AlGamdi *et al.* [7]. Like our results, Almundarij, *et al.,* [10] reported that P- hydroxybenzoic acid, caffeic acid, and ferulic acid were detected in both *L. sativum* and *A. hierochuntica*. Moreover, in our study, catechol was detected in *A. hierochuntica* while it was not detected in *A. hierochuntica* in the study of Almundarij, *et al.* [10]. Presence or absence of the phenolic acids and differences among its concentrations in all studies can be attributed to genotypic and environmental variation within species and choice of tested plant parts [32].

Nature has always served as an immense source for human that the phenolic compounds which have various properties. This part of the current work indicated various properties of benzoic acid, cinnamic acid, rutin and vanillin that act as antimicrobial and antitumor agents. Benzoic acid, one of the phenolic acids, have been found to inhibit the development of some bacteria [33]**.** Our results showed that benzoic acid was more active against tested Gram-negative bacteria and the same results have been reported by Willey *et al.,* and Adeshina *et al.* [37]. Abushady *et al.* [38] reported that, benzoic acid showed antitumor effect on growth of human fibroblasts at the concentration of 0.02% and cell viability at this concentration was below 90%. Also the results obtained by Kahraman *et al.* [39] stated that cinnamic acid, rutin and vanillin have a significant cytotoxicity potential from 24 to 48 hours using the brine shrimps bioassay [40].

5. Conclusion

This study represents the continuity of work from our lab to characterize locally available plants that grow in Egypt. The present investigation provided supportive data for the possible use of *L. sativum* and *A. hierochuntica* in treatment of various diseases. They can also be a rich source of compounds that can then be structurally and chemically modified in drug design for the development of new drugs. Data in current investigation confirmed the presence of biologically active secondary metabolites in both the plants and the extracts of these plants had potential antioxidant, antibacterial and antitumor properties. Therefore, if explored further, these medicinal plants used by the indigenous population of Egypt can not only be used as good alternative for synthetic drugs but also as a promising source for designing bioactive compounds.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of Interest.

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