Bioactivity of three medicinal plants leaf aqueous extracts growing wild in Palestine

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Abstract

The aim of the present study was to investigate some biological activities of Aizoon hispanicum, Heliotropim maris-mourtui and Polygonum arenarium which are wildly growing in Palestine. The first part of the current research was to find out the effect of the screened plant extracts on hepatic GSTs spectrophotometrically. The obtained results revealed that all examined different aqueous extracts from the plant species under study demonstrated inhibitory effect on GSTs activity at all tested concentrations. Among all the investigated plant extracts, result showed that A. hispanicum exhibited the strongest inhibitory effect on GSTs activity.

The second part of this study was to confirm the antioxidant activity of the investigated plant species through DPPH assay. P. arenarium aqueous extract showed the highest antioxidant activity which is relatively similar to the antioxidant power of ascorbic acid. The last part of this study was conducted to evaluate the antibacterial effect of the three extracts against six different bacterial isolates using broth dilution method. The obtained antibacterial results indicated that all examined extracts were able to act against all studied bacteria with observed variation among them. In conclusion, results indicated that the extracts of the tested plant species could be explored as natural remedies for health promotion.

Keywords: Bioactivity, medicinal plants, antioxidants, antibacterial, glutathione-s-transferases activity.

Introduction

Herbal plants have been traditionally utilized in medicine and are still used in healthcare throughout the world due to their valuable chemical constituents. According to The World Health Organization, more than 80% of the world populations in developing countries relies primarily on plants as a source of basic healthcare requirements[43]. In this aspect, the utilization of complementary and alternative medicine in Palestine is prevalent. So, scientists in Palestine try to study the bioactivity of several wild plant species[46]. For that reason, this study was performed to find out the effect of *Aizoon hispanicum, Heliotropium maris-mortui* and *Polygonum arenarium* on glutathione-s-transferases (GSTs). Besides that, to assess the antibacterial and antioxidant activities of these plant extracts.

The species *Aizoon hispanicum* L. is one of the target plants in this study. It is an annual desert plant that belongs to *Aizoaceae* family[19]. It is widespread in the hot and saline Jordan valley and Negev Deserts of Palestine[15]. Moreover, this plant species is used in veterinary medicine to stimulate the milk production[30]. Besides this plant species, *Heliotropim maris-mourtui* Zohary, a member of *Heliotropium* genus that belongs to *Boraginaceae* family, was also studied. *Heliotropium* genus consists of about 300 species which are commonly known as heliotropes and are widely distributed in the tropical and temperate regions of the world[44].

Furthermore, *Heliotropium* species have been broadly used for the treatment of rheumatism, gout and febrifuge. Also, they were used as antiseptic, anti-inflammatory and healing agents[41]. Several studies have previously reported the bioactivity of different *Heliotropium* species in the literature.[2,24] In addition to that, *Polygonum arenarium* Waldst. and Kit was another point of focus in this study. This plant species is one of 300 species related to *Polygonaceae* family distributed worldwide in temperate climates[52]. The phytochemical evaluation of this genus reported the presence of many kinds of polyphenols. Otherwise, it was elucidated through different research that most of these polyphenols demonstrated some biological activities[16]. In addition to that, a series of recent studies has indicated that there are many applicable uses for this species in food and folk medicine[8,32,53,58].

Glutathione-s-transferases isoenzymes are highly distributed in nature as they are found in a wide range of organisms mainly from microbes to mammals[22]. These isoenzymes act as a complex group of proteins which catalyze the conjugation of reduced glutathione (GSH) with substrates that include an electrophilic centre which forms a thioether bond with a sulphur atom of GSH[35]. Moreover, other GSH-dependent catalytic activities are exhibited by GST isoenzymes like the reduction of organic hydroperoxides and isomerisation of various unsaturated compounds[4,25].

Also, these enzymes perform several non-catalytic functions including carcinogens segregation, intracellular transport of a wide spectrum of hydrophobic ligands and modification of signal transduction pathways[1,9,33]. Since some of plant phytochemicals play an active role in inhibition and induction of GSTs activity, many researchers have been focusing on screening the effect of plant extracts on GSTs activity[17,57].

Antioxidant activity is a broadly used term to characterize substances with the ability of scavenging or neutralizing free radicals[29]. The high amount of free radicals causes a phenomenon known as oxidative stress which in turn alters the structure of proteins, lipids, lipoproteins and DNA[50].

Consequently, many disorders may propagate and can lead to degenerative, cardiovascular, renal, neurological, liver and autoimmune diseases[6]. So, to protect cells against this toxic effect, it is required to use diets and plants rich with antioxidant components to neutralize excess radicals.

There are many reports on the antimicrobial activity of plant extracts and their essential oils that could serve as novel sources for antimicrobial agents against different microbes. So, it is necessary to find out the effect of the prepared extracts on bacteria. In this research, the examined bacterial isolates are common pathogens in human diseases. Among them, *Bacillus subtillus* is a ubiquitous bacterium commonly recovered from water, soil, air and decomposing plant residue. This bacterium produces an endospore that allows to tolerate extreme conditions. In rare cases, it is considered as pathogenic bacteria[18]. The other bacterial isolate *Escherichia coli* is an enteric gram-negative bacterium.

Enteropathogenic strains of *E. coli* produce toxins that cause secretory diarrhea, commonly called Travellers' diarrhea[7]. The other studied bacterium is *Klebsiella pneumoniae*. This bacterium is a gram-negative rod that causes bacterial pneumonia and hospital-acquired infections[36]. *Proteus vulgaris* has been reported to cause urinary tract infections, wound infections, burn infections, bloodstream infections and respiratory tract infections also examined[31].

In addition to that, *Staphylococcus aureus* was also studied. *Staphylococcus aureus* is a gram-positive bacterium which is one of the leading causes of human infections of the skin, soft tissues, bones and joints, abscesses as well as normal heart valves[27]. The last studied bacterium is *Staphylococcus epidermis* previously regarded as an innocuous commensal microorganism on the human skin, but now it is the most frequent cause of nosocomial infections[40].

Material and Methods

Plant Collection: Plant species *Aizoon hispanicum*, *Heliotropium maris-mortis* and *Polygonum arenarium* were collected from West Bank, Palestine. They were identified by Ghadeer Omar, Department of Biology and Biotechnology, An-Najah National University; Palestine. Representative plant specimens were pressed till drying, treated chemically, mounted on herbarium sheets and deposited at An-Najah National University herbarium.

Plant Extract Preparation: For extract preparation, leaves of the three plant species were washed with water and air dried in shade at room temperature. After that, the dried leaves were placed in a grinder and crushed to get a fine powder.

The obtained powder was kept in plastic bags in a dark place at room temperature. For aqueous extract preparation, five grams of the prepared powders from each studied plant species (*A. hispanicum*, *H.maris-mortis* and *P.arenarium*) were soaked in 100 ml of warm and sterile distilled water. The aqueous mixtures were subjected to rotary shaking at room temperature for 72 hours. The soaked plant species were macerated by a probe sonicator (3 seconds sonication and 5 seconds rest) for 15 minutes at 25°C. After that, the mixtures were centrifuged for 10 minutes at 4500 rpm. Then, the obtained supernatants from all extracts were filtrated and evaporated by freeze-drying. The extracted powder of each plant species was dissolved in sterile distilled water forming a stock working solution with a final concentration equal to 100 mg/ml for antimicrobial assay and 1000, 750, 500, 250, 100 µg/ml for GSTs activity and antioxidant assay.

Glutathione-S-Transferases Activity Assay

Glutathione-S-Transferases Purification: Sheep liver glutathione-s-transferase was obtained from the Protein Purification Laboratory, Biology and Biotechnology Department, Faculty of Science, An-Najah National University. The enzyme solution was purified as follows: fresh sheep liver was washed, homogenized and centrifuged. The resulting supernatant that contains cytosolic glutathione-s-transferases was precipitated by ammonium sulfate at 30-70 %. Subsequently, the obtained ammonium sulfate fraction was further purified by gel filtration column chromatography using (Ultrogel ACA 44 column, Sigma).

The protein levels for all fractions and GSTs activity for protein containing fractions were estimated[23,54]. Then, all fractions with GSTs were collected and applied to affinity column (GSH-agarose, Sigma). After elution, all fractions were examined to determine GST activity and protein level[23,54]. The fractions with GSTs were collected, dialyzed, concentrated by freeze-drying to a concentration equal to 200 µg/ml and used for the activity studies.

Glutathione-S-Transferases Assay under Different Concentrations of the Plant Extracts: Glutathione-S-transferases activity was carried out spectrophotometrically using a substrate 1-chloro- 2, 4- dinitrobenzene (CDNB) as a substrate [23]. The cuvettes contained 0.2 M phosphate buffer (pH=7), 1.5 mM GSH, 1.5 mM of CDNB and 50 µl of diluted enzyme (0.5 µg/ml) in a final volume of 1000µl. Change in absorbance at 340 nm was followed against a blank containing all reactants except CDNB. The GSTs activity was expressed as µmol conjugate formed/min/ml using a molar extinction coefficient of 9.6 $mM^{-1}.cm^{-1}$.

The effect of the plant extracts at different concentration (250, 500, 750 and 1000µg/ml) was measured by the addition of 50 µl from each extract concentration to the enzyme reaction mixture. Then the effect of each extract concentration on the activity of GSTs was measured by Seacoman spectrophotometer at 340nm and expressed as inhibition percentage.

Inhibition % = GSTs activity without treatment – GSTs activity under treatment of specific plant extract concentration / GSTs activity without treatment × 100 %

Antioxidant Assay: 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical assay was carried out in a 96-well microplate [36]. Briefly, 100 µl of various concentrations of extract in methanol (3.125-100 µg/ml) was added to 100 µl of 0.01% methanolic DPPH solution. The plate was incubated for 30 min in the dark at ambient temperature and the absorbance was recorded at 540nm using a spectrophotometer (microplate reader). Ascorbic acid at different concentrations (3.125-100 µg/ml) was used as standard. The DPPH radical scavenging activity (%) was calculated as follows:

DPPH scavenging activity (%) = [(A control – A sample) / A control] × 100 %

Antibacterial activity Assay

Studied Bacteria: *In vitro* antibacterial activity of the plant extracts was evaluated against a total of six bacterial isolates which includes three gram negative bacteria, *Proteus vulgaris* (ATCC# 8427), *Klebsiella pneumoniae* (ATCC# 13883) and *Escherichia coli* (ATCC# 25922). In addition to three gram positive bacteria, *Staphylococcus aureus* (ATCC# 6538P), *Staphylococcus epidermis* (ATCC# 12228) and *Bacillus subtillus* (ATCC# 6633) are present.

Micro-broth Dilution Method: The tested bacteria were grown overnight on nutrient agar plates. Broth turbidity was adjusted to 0.5 McFarland (1.5×10^8 CFU/ml). Then it was diluted in saline to obtain 1×10^7 CFU/ml. All plant extracts were examined for their minimum inhibitory concentration (MIC) by micro-broth dilution method [39]. The prepared extract was serially diluted two fold in the nutrient broth medium. Duplicates of each dilution (50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391, 0.195 and 0.098 mg/ml) were inoculated with 1µl of 1×10^7 CFU/ml. The last two duplicate wells were not inoculated and considered as negative controls.

After that, the inoculated microtiter plates were incubated at 37°C for 18 hours. The lowest extract concentration (highest dilution) that inhibited the growth of tested microorganisms was considered as MIC. Then, the contents of the wells resulting from MIC were streaked using sterile cotton swaps on agar plate free of antibacterial agents and incubated at 37°C for 18 hours.

Finally, the lowest concentration of the extract which showed no bacterial growth was considered as minimum bacterial concentration (MBC).

Results

Effect of studied plant extracts on GSTs activity: The first part of the current research was to find out the effect of the screened plant extracts on hepatic GSTs. The obtained results revealed that all examined different aqueous extracts from plant species under study demonstrated an inhibitory effect on GSTs activity at all tested concentrations (250, 500, 750 and 1000 µg/ml). Among all the investigated plant extracts, result showed that *A. hispanicum* exhibited the strongest inhibitory effect on GSTs activity mainly at 1000 µg/ml concentration with 41.11% inhibition percentage (Figure 1).

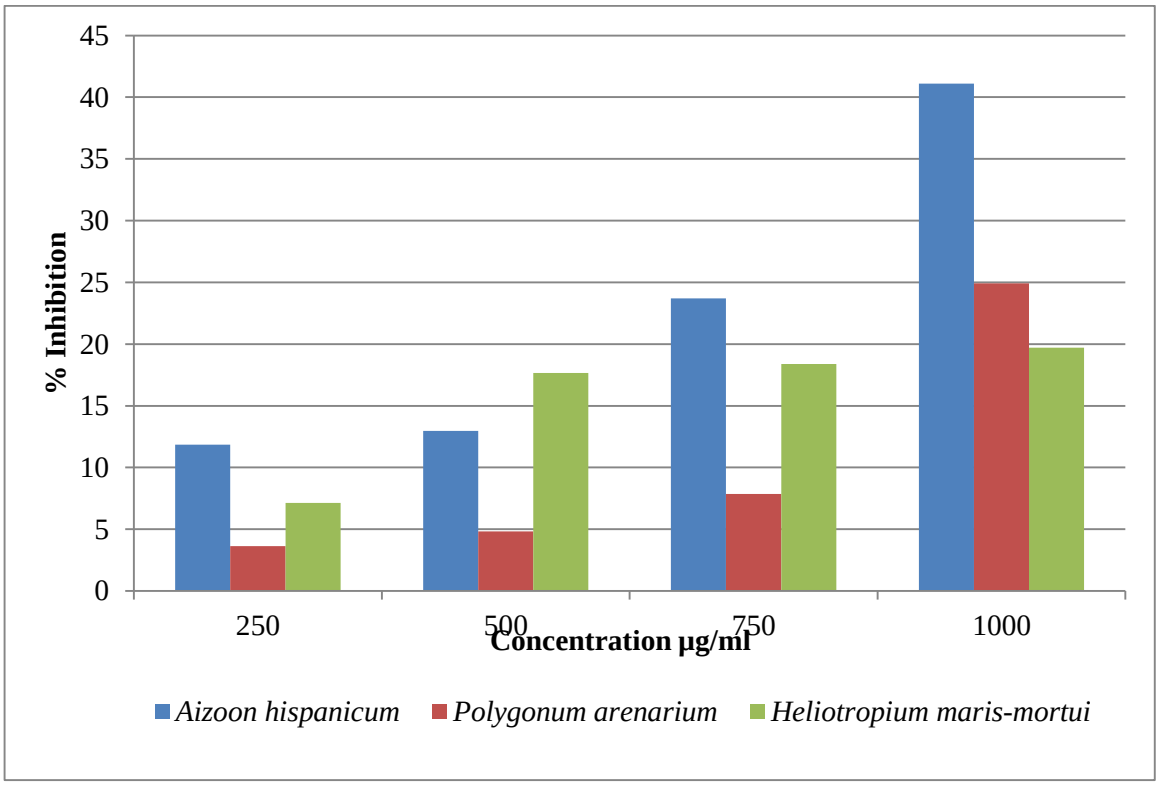

Fig. 1: GSTs inhibitory percentage (%) by *A. hispanicum, H. maris-mortis* and *P. arenarium* aqueous extracts

On the other hand, the aqueous extracts from *H. maris-mortui* and *P. arenarium* showed a low inhibitory effect on GSTs activity when compared to *A. hispanicum* extract. Given that, the inhibition percentage for both plant extracts at 1000 µg/ml reached 24.91% and 19.71% respectively. On the top of that, all examined plant species aqueous extracts inhibited GSTs activity in a concentration dependent manner.

Antioxidant activity of plant extracts: DPPH assay is routinely employed in laboratories for determining the free radical scavenging potential of natural plant extracts. The obtained results revealed that all examined different aqueous extracts from plant species under study showed antioxidant activity (Figure 2). *P. arenarium* aqueous extract showed the highest antioxidant activity that is relatively similar to the antioxidant power of ascorbic acid. *H. maris-mortui* illustrated a moderate antioxidant power when compared to the ascorbic acid and *P. arenarium*. Otherwise, the aqueous extract from *A. hispanicum* showed a low percentage of inhibition of DPPH activity.

Antibacterial activity of plant extracts: The third part of this study was conducted to evaluate the antibacterial effect of the three aqueous extracts against three gram-negative and three gram-positive bacterial isolates. The antibacterial activity of the plant species aqueous extracts was quantitatively recorded by the measurement of their MIC concentrations against the examined bacterial isolates using micro-broth dilution method (Figure 3). The MIC results indicated that all examined extracts were able to act against all studied bacteria with the observed variation among them. This elucidated the broad spectrum antibacterial behavior of the three extracts.

Moreover, the aqueous extract of *A. hispanicum* exhibited the best inhibitory behaviour among the other ones. It inhibited the growth of all tested bacterial isolates in a concentration range between 1.563 mg/ml and 25 mg/ml. Furthermore, the lowest MIC value (1.563 mg/ml) was recorded for *A. hispanicum* extract against *K. pneumoniae*. However, *P. arenarium* extract displayed moderate bacteriostatic potential with a concentration range (6.25 mg/ml-25 mg/ml) while *H. maris-mortui* inhibitory activity was the lowest with a limited concentration range between 25 mg/ml-50 mg/ml.

In addition to that, the bactericidal effect of all plant extracts that exhibited an inhibitory effect was determined by measuring their minimum bactericidal concentration (MBC) (Figure 4). The obtained MBC results confirmed that the aqueous extract from *A. hispanicum* was the most potent one as it had a bactericidal effect at concentration range (12.5 mg/ml-50 mg/ml).

Discussion

Glutathione-s-transferases enzymes are involved in the detoxification of many xenobiotics and endogenous compounds in addition to their role in prostaglandins production[51]. According to the literature, GSTs can interact with plant-derived extracts as some can inhibit their activity and others induce it.

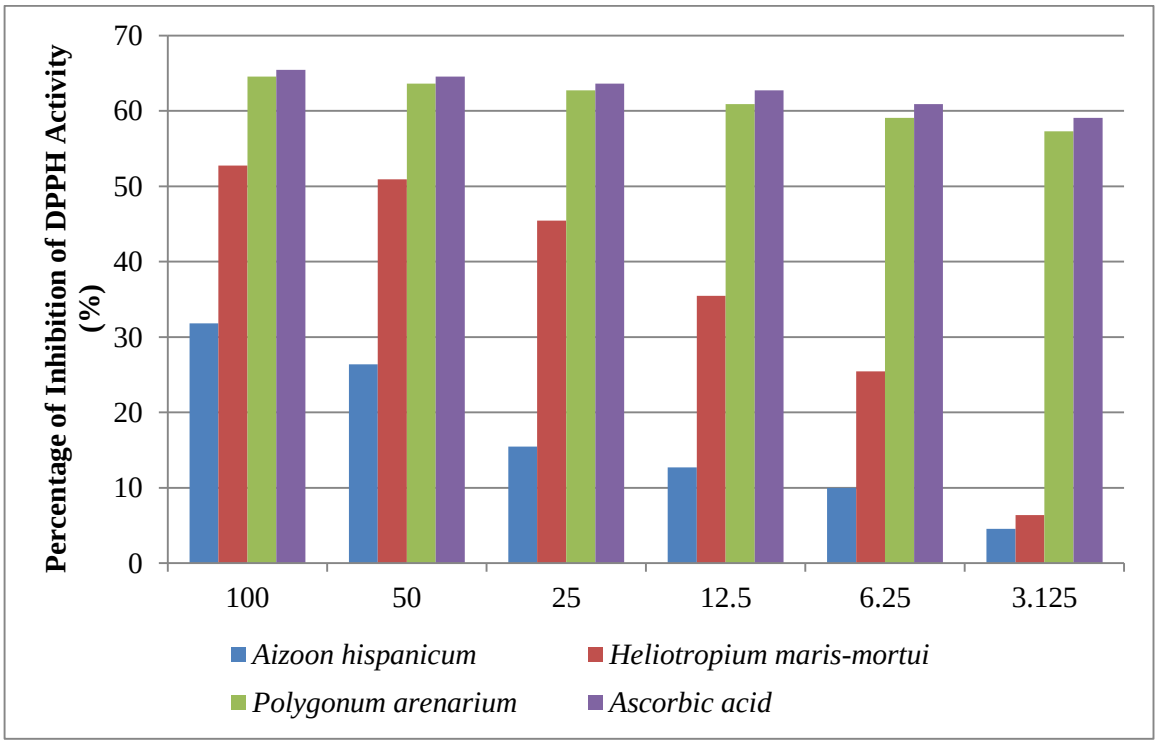

Fig. 2: Percentage of inhibition of DPPH activity of the aqueous extracts from *A. hispanicum, H. maris-mortis* and *P. arenarium*

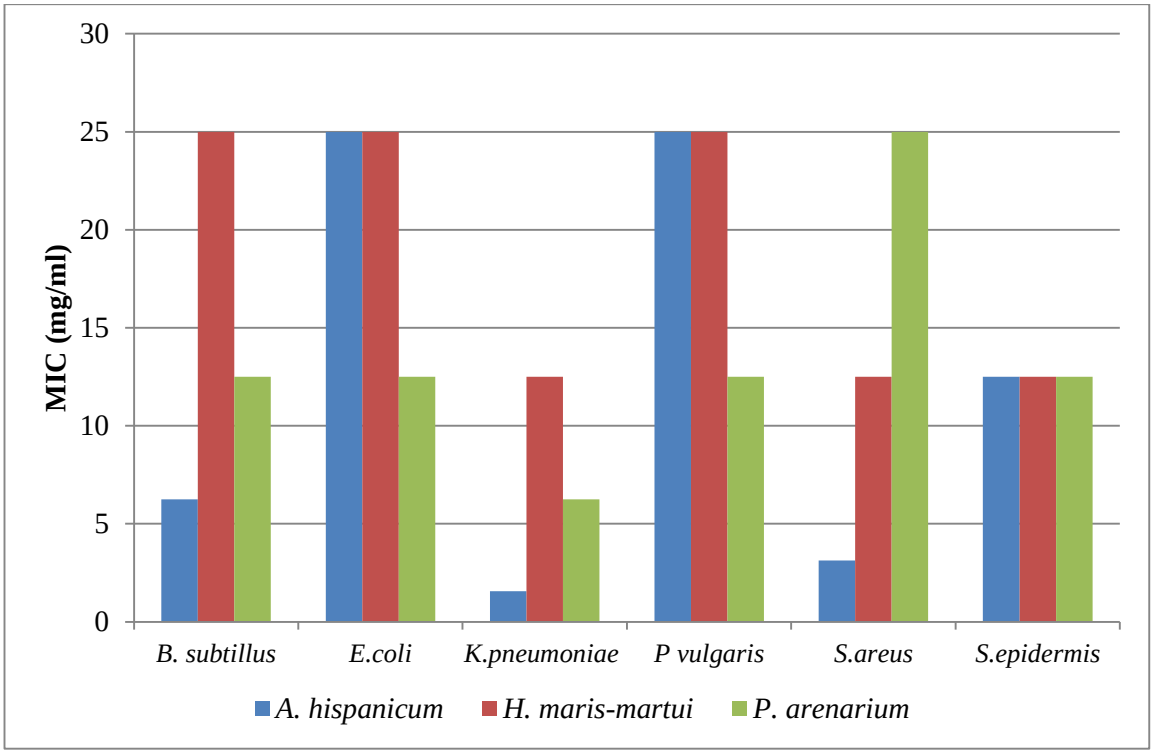

Fig. 3: Antibacterial activity of *A. hispanicum, H. maris-mortui* and *P. arenarium* against *B. subtillus, E. coli, K. pneumoniae, P. vulgaris, S. areus* and *S. epidermis* bacterial isolates using micro-broth dilution method, (MIC) minimum inhibitory concentration (mg/ml)

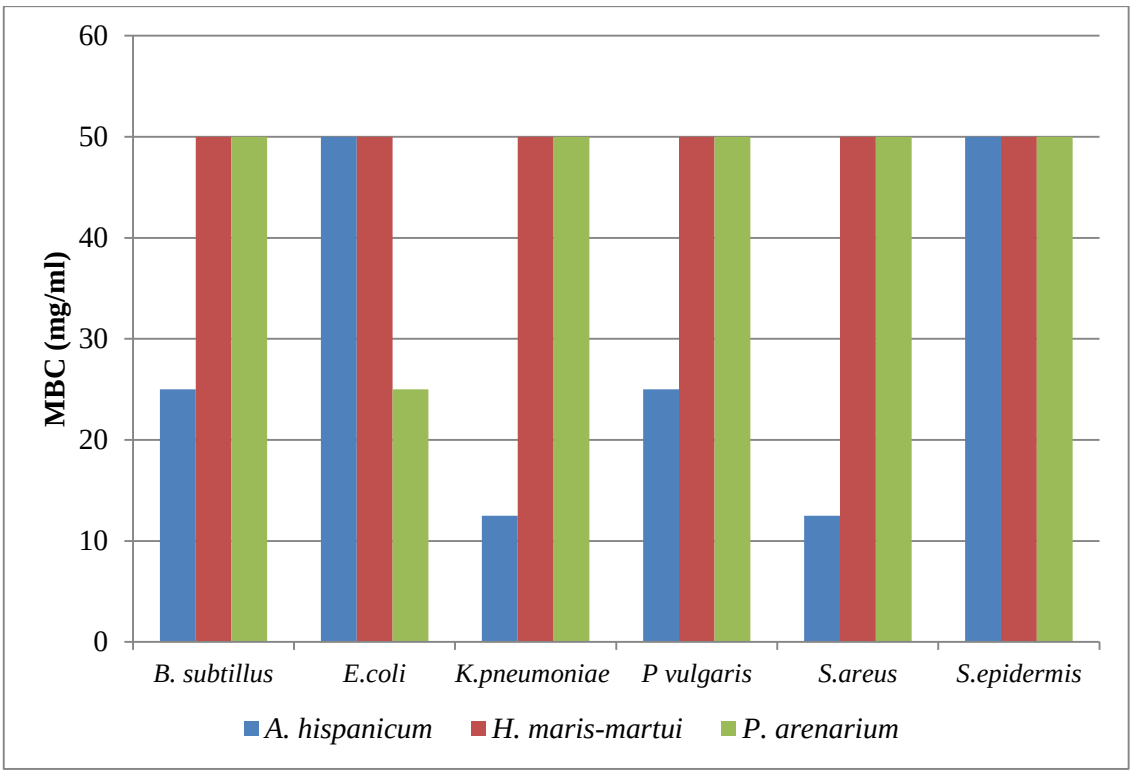

Fig. 4: Antibacterial activity of *A. hispanicum, H. maris-mortui* and *P. arenarium* against *B. subtillus, E. coli, K. pneumoniae, P. vulgaris, S. areus* and *S. epidermis* bacterial isolates using micro-broth dilution method, (MBC) minimum bactericidal concentration (mg/ml)

Thus, the inhibition of these isoenzymes can cause harm as the detoxification of electrophilic compounds is decreased. Therefore, it can harm DNA, lipids and proteins, then ends up with tumors and neurodegenerative disorders[51]. On the other hand, the induction of GSTs may be beneficial in protecting the cells from electrophilic damage. This damage

can cause cancer and neurodegenerative diseases by taking electrons from macromolecules like DNA and proteins[51].

Nevertheless, it is favorable to inhibit GSTs activity once they are over expressed in some kinds of tumors. Consequently, this over expression of GSTs leads to the formation of drug-resistant tumors[38]. According to the former knowledge, GSTs metabolize the drug and making it more soluble and enhancing its excretion from the body. As a result, this leads to the reduction of the drug residence time in the body and then therapeutic failure [48]. So, it is useful to use GSTs inhibitors in such cases of tumors to increase the sensitivity of cancer cells to antitumor drugs.

Additionally, this inhibitory effect may be applied to oxidative stress, cell proliferation and parasite treatments such as malaria and schistosomiasis as well as for insecticide resistance[13,34]. Based on that, the inhibition of GSTs has been extensively studied *in vitro* through many approaches. A common one is studying the effect of plant compounds on the activity of GSTs.

In that case, researchers found that many natural polyphenols from plants are inhibitors of GSTs enzymes. These natural compounds include tannic acid, thonningianin A, cibacron blue, hematin, ethacrynic acid, ellagic acid, ferulic acid, caffeic acid, stilbene, quercetin, chlorogenic acid and curcumin[21,28,55]. In this experiment, *A. hispanicum* aqueous extract exhibited the best rate of enzymatic inhibition (41% inhibition) despite the low content of phenolics and flavonoids[30] while other examined plant aqueous extracts were not as desired.

Take into consideration that the ethanol solution probably will give a higher rate of enzymatic inhibition owing to the fact that the solubility of polyphenols in alcoholic solutions is better than in aqueous ones[20]. Besides that, it was approved that the secondary metabolite content of plants can differ greatly due to the harvesting time and the environmental conditions[10]. For all that, more chemical analysis and *in vivo* experiments must be carried out to assess the inhibitory effects of all studied plant species in this research before considering them as therapeutic agents for diseases such as cancer.

In DPPH free radical scavenging activity experiment, *A. hispanicum* gave the lowest percentage of DPPH inhibition. This is mostly due to its low content of flavonoid and phenolic compounds[30]. Additionally, *H. maris-mortui* extract worked well at high concentrations and its effect decreased gradually at low concentrations. On the other hand, *P. arenarium* gave a greater significant inhibitory effect that almost matches the effect of ascorbic acid at all studied concentrations. This may be related to its high content of flavonoid and phenolic compounds[3]. Previous studies have emphasized that flavonoids and phenolics are generally involved in protecting plants from UV and known as radical free scavengers[12,42]. Accordingly, they are excellent natural sources of antioxidants, which can be used for the development of herbal drugs[5].

Antibacterial screening showed that the prepared plant extracts were effective against all tested microorganisms at different concentrations. The aqueous extract of *A. hispanicum* revealed a wide antibacterial spectrum against most tested bacterial isolates with low MIC and MBC values. This extract inhibited the growth of all studied bacterial isolates at a concentration range between 1.563 and 25 mg/ml. Notably, the obtained data in the running research agreed with some previous studies[37]. Actually, the obtained results can be explained by the presence of various classes of chemical compounds in Aizoaceae family. These phytochemicals include alkaloids, phenolic compounds, betacyanins, triterpenes, sterols, lignans and essential oils[56].

In the same aspect, *H. maris-mortuli* aqueous extract demonstrated moderate antibacterial activity against *S. aureus*, *S. epidermis* and *K. pneumoniae*. This observation can be attributed to the presence of metabolites like alkaloids, saponins and pyrrolizidine in this plant genus. Over that, the antibacterial activity of *Heliotropium* genus has been previously documented [49]. Furthermore, *P. arenarium* exhibited antibacterial activity higher than *H. Maris-mortuli* and lower than *A. hispanicum.* This plant extract manifested a wide spectrum antibacterial agent against all screened bacteria.

In fact, plants are an important source of potentially useful compounds for the development of new chemotherapeutic agents. To point out that *in vitro* antibacterial activity of plant extracts was the first step toward this goal [47]. Therefore, the results of this study provide evidence that some medicinal plants might indeed be potential sources of new antimicrobial compounds to the resistant strains. In this study, it was observed that the extracts of the plant species had antibacterial activity against all tested Gram positive and gram negative bacteria.

Moreover, the obtained results provided that these plant extracts were more active against gram positive bacteria. This antibacterial activity coincides with many previous studies[11]. The pronounced effect of several plant extracts on gram positive bacteria can be explained by the fact that the gram negative microorganisms are less susceptible to the active compounds in comparison to the gram positive ones. Hence, gram negative bacteria possess outer membranes surrounding their cell walls which restrict diffusion of such compounds[45].

Conclusion

In general, plants have an almost limitless ability to synthesize aromatic substances which serve as plant defense mechanisms against predation by microorganisms, insects and herbivores[14]. Nevertheless, data from the previous literatures as well as the obtained results revealed that plants offer a great potential for disease therapy. Therefore, it is

recommended to search, purify and characterize new compounds from the investigated plant species. Further studies are necessary to explore the use of these plant extracts and their new compounds as safe alternatives to synthetic drugs.

Acknowledgement

We would like to thank Biology and Biotechnology Department at An-Najah National University for allowing us to access their facilities. Thanks also go to Dr. Ghadeer Omar for her role in plant classification.

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(Received 10th September 2020, accepted 14th November 2020)