Turkish Journal of Agriculture - Food Science and Technology, 11(2): 383-389, 2023
DOI: https://doi.org/10.24925/turjaf.v11i2.383-389.5362

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Antioxidant, Antibacterial Activities and Synergistic Effect with Antibiotics of Fruits and Stems Extracts from *Styphnolobium japonicum* (L.) Schott

Karima Saffidine[1,a,*], Fatima Zerargui[1,b], Thoraya Guemaz[1,c], Charafeddine Lameche[2,d], Farida Sahli[3,e], Abderahmane Baghiani[1,f]

[1]*Laboratory of Applied Biochemistry, Faculty of Nature and Life Sciences, Ferhat Abbas University Setif1, Setif, Algeria.*
[2]*Department of Pharmacy, Faculty of Medicine, Ferhat Abbas University Setif 1, Setif, Algeria.*
[3]*Laboratory of Bacteriology, CHU of Setif, Setif, Algeria.*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 05-07-2022
Accepted : 03-10-2022

Keywords:
Antimicrobial activity
Antioxidants
Flavonoids
Polyphenols
Styphnolobium japonicum

ABSTRACT

The objective of this study was to evaluate the antioxidant and antibacterial properties of *Styphnolobium japonicum* (L.) Schott extracts. Results showed that the total phenols and flavonoids contents of the extracts ranged from 24.57 to 316.11 mg GAE/g and from 1.80 to 12.41µg QE/mg, respectively. Ethyl acetate (EaES) and aqueous (AqES) extracts from seeds showed the highest DPPH scavenging capacity (IC_{50}= 5.51 and 23.43 µg/mL). EaES from seeds, stem bark and fruit pod were the most active in inhibiting β-carotene oxidation. While, aqueous extracts have the ability to chelate ferrous ions. Significant antibacterial activity was obtained against *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Bacillus cereus* and *Pseudomonas aeroginosa;* and inhibition zones varied between 10 to 29.5 mm. MIC and MBC ranged from 0.7–11.25 mg/mL and 5.63–22.5 mg/mL, respectively. In addition, synergistic effects were obtained, where inhibition zones varied between 7 and 50 mm. These results support the use of this species in traditional medicine in many physiological disorders and could be used where antioxidant and antibacterial are warranted.

[a] *saffidinekarima@gmail.com* *https://orcid.org/0000-0001-7122-961X*
[b] *fatimazerargui512@gmail.com* *https://orcid.org/0000-0002-4059-3600*
[c] *thoriague@yahoo.fr* *https://orcid.org/0000-0002-5074-3991*
[d] *lameche@hotmail.com* *https://orcid.org/0000-0003-0014-4701*
[e] *sahlimrnicrobio@yahoo.fr* *https://orcid.org/0000-0001-9858-0530*
[f] *baghiani@yahoo.fr* *https://orcid.org/0000-0002-6881-011X*

Introduction

In recent years, there has been a considerable interest in finding natural antioxidant and antimicrobial agents from medicinal plant, which are relatively safer than synthetic alternatives (Sevindik et al., 2017; Saratale et al., 2018; Mohammed et al., 2022). Antioxidants help to scavenge free radicals whose excessive production can cause damage to macromolecules (protein, lipid and DNA) and are involved in cardiovascular diseases, cerebral disorders and cancer (Liguori et al., 2018; Moldogazieva et al., 2018; Akgül et al., 2022; Unal et al., 2022). Free radicals are also involved in the oxidation of lipids in food products reducing their nutritional quality (Domínguez et al., 2019; Krupodorova et al., 2022) and may be associated with cancer risks. Many studies have shown that polyphenolic compounds and flavonoids react as reducing agent, free radicals quenchers and metal chelating agent (Salehi et al., 2020; Pehlivan et al., 2021). Moreover, the emergence of bacteria and fungi resistant to many antibiotics is actually a public health. Nowadays, current researches are looking for new alternative treatments of plant origin, which can be exploited in agri-food, cosmetic and pharmaceutical industries (Kına et al., 2021; Uysal et al., 2021).

In this context, the present paper reports the evaluation of the antioxidant and antimicrobial activities of *S. japonicum* extracts. It is an attractive ornamental tree with fragrant creamy white flowers in summer, a shrub species belonging to Fabaceae family. Since antiquity, different parts of this plant (flowers, fruits and buds) were used to dye silk in yellow. This species is widely known for its use in traditional Chinese for therapeutic purposes. Decoction of the young stems is used to treat eye congestion, photophobia, scabies, eczema and leucorrhea (Han et al., 1996; Ran, 1998). Stem bark of *S. japonicum* is also used to treat inflammatory diseases and as an analgesic agent (Wang et al., 2006; Zhang et al., 2011). The dried flowers and buds have been used to treat bleeding hemorrhoids, dysfunctional uterine bleeding, hematemesis and diarrhea (Kim, 2008; Ha et al., 2010). It has also been shown that the fruit extract

possesses beneficial effects on postmenopausal symptoms in women (Lee et al., 2013), and the leaves reduce the blood pressure and inflammation (Kim et al., 2004). In Algeria, this plant namely called "Sephira or Sophera" which refers to a tree giving a yellow dye and also cultivated as an alignment or ornamental tree in large gardens or parks. According to our enthnobotanical study around Setif area, the decoction of the bark stems is reputed to treat diabetes.

Materials and Methods

Plant material

S. japonicum fruits and bark stems were collected in January 2018 from Setif area, eastern Algeria. The Plant was authenticated by Pr Chermat Sabah, Department of Pharmacy, Faculty of Medicine and Ferhat Abbas University of Setif, Algeria. The fresh plant parts were cleaned, shade dried at the room temperature during two weeks and grinded into powdered form.

Bacterial strains

Antibacterial tests were carried out using referenced strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeroginosa* (ATCC 27853) and clinical origins strains: *Acinetobacter baumanii*, *Serratia* sp obtained from the Laboratory of Bacteriology at Sétif hospital. *Bacillus cereus* (ATCC 10876), *Enterobacter faecalis* (ATCC 49452), *Salmonella typhimurium* (ATCC 13311), *Citrobacter freundii* (ATCC 8090), *Proteus mirabilis* (ATCC 35659) and *Klebsiella pneumonia* (ATCC 700603) were obtained from Laboratory of Natural Substances at the University of Tlemcen, Algeria.

Extraction process

Bark of stems (70g), fruit pods (50g) and seeds (100g) were respectively macerated (1/10) in hydro-methanol (3/7) for 24 hours according to Saffidine et al. (2015). Each mixture was then filtered on filter paper and the recovered marc was macerated twice again. The filtrates were collected and evaporated to dryness under reduced pressure at 40°C, with a rotary evaporator. Each dry residue was treated with boiling distilled water, let settle in the refrigerator for 24 hours and filtered through filter paper. The aqueous solution of stem bark and fruit pod was firstly extracted with hexane to remove lipids, then with ethyl acetate. Two organic phases: hexane fruit extract (HeEF), hexane bark extract (HeEB), ethyl acetate fruit (EaEF) and ethyl acetate bark (EaEB) fractions were obtained. Residual aqueous phases were AqEF and AqEB for the fruit pod and stem bark, respectively. In the case of seeds, only ethyl acetate was used and two fractions were obtained: an organic (EaES) and an aqueous (AqES) phases. These fractions were evaporated to dryness under reduced pressure to 45°C, with a rotary evaporator and stored in the fridge.

Determination of total phenol content

Extracts total phenol was determined using Folin-Ciocalteu assay (Guemmaz et al., 2018). 200 µL of sample (concentration 0.5 mg/mL) or Gallic acid as standard (0 to 200 µg/mL), was mixed with 1 mL of dilute Folin-Ciocalteu reagent (1/10). After 4 min, 800 µL of aqueous sodium carbonate solution (7.5%) was added. Samples were incubated in the dark at room temperature for 2 h and data were recorded at 765 nm. The results were expressed as milligram gallic acid equivalent per gram of dry extract (mg GAE/g extract).

Determination of total flavonoids

Total flavonoids content of extracts was estimated using aluminum chloride (AlCl3) as described by Adjadj et al. (2017). 1mL of extract (2 mg/mL) or Quercetin standards (0 to 40µg/mL) was combined with one mL of AlCl3 solution (2% in methanol). The mixture was kept in the dark for 10 min at ambient temperature and the absorbance was measured at 430 nm. Results were expressed as milligram quercetin equivalent per gram of dry extract (µg QAE/mg extract).

Antioxidant activities

DPPH scavenging assay

Free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed according to Zerargui et al. (2015), by mixing 50 µL of different dilutions of the extract or standard with 1250µL of DPPH dissolved in methanol (0.004 %). After shaking, the reaction was placed safe from light during 30 min and absorbance was monitored at 517 nm. The synthetic antioxidant butyl hydroxyl toluene (BHT) was used as positive control. The radical scavenging activity (RSA) was calculated using the following formula:

$$\% \text{ RSA} = [(A_{DPPH} - A_{sample})/\text{ADPPH}] \times 100$$

Were %RSA is the percentage of inhibition, A_{DPPH} is the absorbance of DPPH (t=0min) and A_{sample} is the absorbance of the extract (t=30min). IC_{50} expresses the concentration of antioxidant that causes 50% loss of activity of DPPH.

Bleaching β-carotene test

0.5 mg of β-carotene dissolved in 1 mL of chloroform was placed into a flask containing 25 µL of linoleic acid and 200 mg of Twin 40. Chloroform was removed using a rotary evaporator under reduced pressure at 40°C and 100 mL of oxygenated distilled water was then added to the emulsion and shaken vigorously. Aliquots of 2500 µL of this mixture were mixed to 350 µL of the extract or standard (BHT) dissolved in methanol at a concentration of 2 mg/mL (Aouachria et al., 2017). The blank was prepared by mixing 350 µL of extract to 2500 µL of methanol instead the emulsion. Readings of all samples were taken immediately t=0, 1, 2, 4, 6, 24 hours respectively at 490 nm. Antioxidant capacities of the samples were compared with that of butyl hydroxyl toluene (BHT).

The antioxidant activity (AA%) of the extracts was calculated from the equation given below:

$$AA\% = (A_{sample}/A_{BHT}) * 100$$

Where A_{BHT} is the absorbance of the positive control BHT and A_{sample} is the absorbance of the extract.

Ferrous ion chelating activity

This activity was measured by inhibition of the formation of iron (II)-ferrozine complex after treatment of the extracts or standard with Fe2+Ferrous ion. 500 µL of standard or extract were added to 100 µL of $FeCl_2$ (0.6 % µmol in water) and 900 µL of methanol. The mixture was

shaken and allowed to react at room temperature for 5 min. Then, 100 µL of ferrozine (5% µmol) was added to this solution and shaken again (Trabsa et al., 2020). After 10 min of incubation, the absorbance of the solution was measured at 562 nm. The chelating effect was calculated as a percentage of inhibition of ferrozine- Fe^{2+} complex formation, given by the formula:

% inhibition = [(Ac–As)/Ac] ×100

Where Ac was the absorbance of the control and As was the absorbance of the sample. Chelating activity was expressed as IC_{50}, the concentration that chelate 50% of Fe2+ ions.

Antibacterial susceptibility test

Antibacterial activity

Antibacterial activity was screened *in vitro* using the disc diffusion and micro-dilution methods (Saffidine et al., 2015). Sterile Muller-Hinton agar plates were inoculated by the method of streak with inoculums of bacterial suspension from young colonies of 18 to 24 h. For each strain, a suspension was made in sterile distilled water and its turbidity was adjusted to 0.5 McFarland (10^8 CFU/mL). Then, sterile paper discs of 6 mm diameter were impregnated with 20 µL of plant extract, dried and delicately deposited on the medium. Each extract was tested in triplicate with the presence of sterile disc impregnated with methanol as a negative control. Standard antibiotic discs Ceftriaxone (CRO) is used as positive control for *B. cereus*, *E. coli*, *K. pneumoniae*, *P. mirabilis* and *S. typhimurium*. Oxacillin (OXA), Cefotaxime (CTX) and Ceftazidime (CAZ) were tested respectively on *S. aureus*, *L. monocytogenes* and *P. aeroginosa*. Plates inoculated with bacteria were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the zone of inhibition against the test microorganism.

Determination of minimum inhibitor concentration (MIC) and Minimum Bactericidal Concentration (CMB)

Micro broth dilution method was used to determine minimum inhibition concentrations (MIC) for extracts showing positive antibacterial activity (CLSI, 2015). For each extract, serial two-fold dilutions were made in the 10 wells of sterile microplate containing nutrient broth Muller Hinton (25 µL). The inoculums of the bacterial strains were prepared from young bacterial cultures of 18 to 24 h and suspension turbidity was adjusted to 0.5 McFarland (10^8CFU/mL). These suspensions were then diluted (1/10) and 5µL of it were inoculated into all wells except the 12^{th} column. This later served as negative control and the 11^{th} as growth control. Then, 70µL of Muller Hinton medium was added to 96 wells and the final density of the inoculum was 5×10^5 CFU/mL. After an overnight incubation at 37°C, the turbidity of the wells in the micro titer plate was interpreted as visible growth of microorganisms. The MIC was defined as the highest dilution or least concentration of the extracts that prevents visible growth of a microorganism. From the wells showing no visible growth, 2 µL were taken and seeded in parallel streaks of 3 cm on the Muller-Hinton agar. After incubation of the plates at 37°C for 24 h, the number of bacterial colonies on the streaks was compared to that of the control plate seeded with streaks of the diluted inoculums of the tested bacteria (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}). The lowest concentration of the extract solution that killing 99.9% of the bacterial inoculums after 24 hours incubation at 37°C was taken as the minimum bactericidal concentration (MBC).

Determination of synergistic antibacterial activities of S japonica extracts and standard antibiotic

The assay was done using the same method of the disc diffusion cited previously. To evaluate the synergistic effect, combinations of *S. japonicum* extracts with standard antibiotics (cefotaxime, ceftriaxone and oxacillin) were tested for the sensitive bacteria. The antibiotic discs were impregnated with 20 µL of plant extract, let evaporate in an oven and then deposited on the medium inoculated with the tested bacteria. The plates were incubated at 37°C for 24 h and results were recorded by measuring the inhibition zone differences around the discs of single standard antibiotic or combined with plant extract.

Statistical analysis

All assays were carried in triplicates and results expressed as means ± standard deviation. Statistical Analysis was carried out by Graph Pad Prism 5. The analysis of variance was determined by one-way ANOVA and differences among the means were determined for significance at $P<0.05$ using Tukey test.

Results and Discussions

Total phenolic and flavonoids content of S japonica extracts

The yields of extracts from the various organs of *S. japonicum* were 20.7%, 22.75% and 17% of seeds, stem bark and fruit pod dry weight, respectively. The results indicated a high variability in the phenolic contents in the different extracts (Table 1). The highest amounts of total phenols were obtained with EaEB, EaES and EaEF (316.19 ± 1.43, 310.16 ± 1.17 and 271.71 ± 1.1 mg GAE/g, respectively). They were higher than the flower phenolic content of the same plant reported by Wang et al. (2006). The same fractions: EaEB, EaES and EaEF contained also high levels of total flavonoid.

Table 1. Total phenolic and flavonoids contents of seed (EaES, AqES), stem bark (HeEB, EaEB, AqEB) and fruit pod (HeEF, EaEF, AqEF) extracts from *S. japonicum*.

Extracts	Total phenols mg EAG/g	Flavonoids µg EQ/mg
HeEB	24.57 ± 0.79	1.80 ± 0.02
EaEB	316.19 ± 1.43	12.41 ± 0.63
AqEB	151.62 ± 2.43	5.71 ± 0.38
EaES	310.16 ± 1.17	10.72 ± 0.04
AqES	104.99 ± 5.74	4.90 ± 0.34
HeEF	45.35 ± 0.39	1.63 ± 0.06
EaEF	271.71 ± 1.1	7.66 ± 0.19
AqEF	45.31 ± 1.09	1.57 ± 0.02

Values were expressed as means ± SD (n=3)

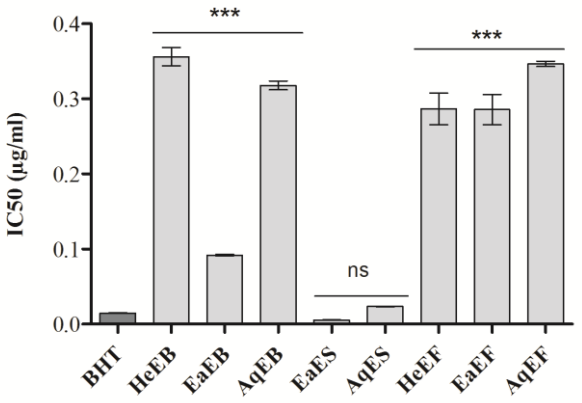

Figure 1. IC50 of antiradical DPPH activity of seed (EaES, AqES), stem bark (HeEB, EaEB, AqEB) and fruit pod (HeEF, EaEF, AqEF) extracts from *S. japonicum*.
Means ± SD = values with standard error of means of three measurements. Comparison with standard (BHT), ns: no significant difference (P>0.05), *** P<0.001.

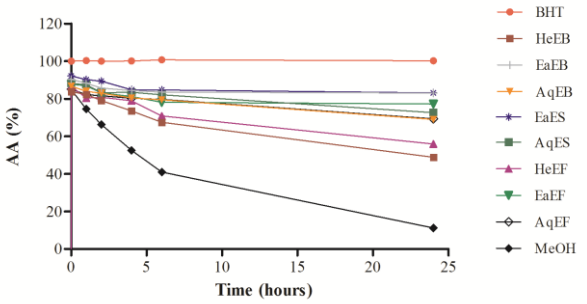

Figure 2. Relative antioxidant activity of seed (EaES, AqES), stem bark (HeEB, EaEB, AqEB) and fruit pod (HeEF, EaEF, AqEF) extracts from *S. japonicum*; standard (BHT) and negative control (MeOH) in β-carotene/linoleic acid assay.
Values are expressed as means of three measurements.

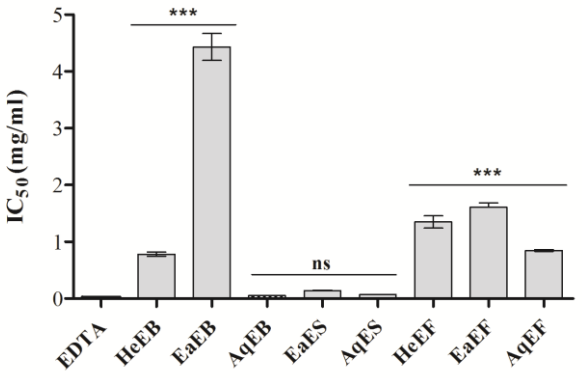

Figure 3. Ferrous chelating IC50 of seed (EaES, AqES), stem bark (HeEB, EaEB, AqEB) and fruit pod (HeEF, EaEF, AqEF) extracts from *S. japonicum*.
Means ± SD (n = 3). Compared to EDTA as standard, ns: no significant difference P>0.05, *** P<0.001.

Antioxidant activities

DPPH radical scavenging activity

In DPPH radical scavenging assay, all extracts exhibited scavenging activities in a concentration dependent manner. As shown in Figure 1, the seed fractions (EaES and AqES) possessed a strong antioxidant activity with an IC_{50} = 5.51 ± 0.18 and 23.43 ± 0.29 µg/mL and were more effective than the positive control BHT. Our results are in agreement with the result reported by Park et al. (2009) and Wang et al. (2006), but different from that reported for flower extract by Zang et al. (2011). A significant antioxidant of 91.99 ± 0.98µg/mL was exhibited by EaEB. It is interesting to note that several flavonoids have been isolated from *S. japonicum* like rutin, quercetin, isorhamnetin, genistein and kaempferol (Ma et al., 2007; Cai et al., 2004); known to be potent radical scavenger (Russo, 2018).

Bleaching β-carotene assay

Determination of antioxidant activity using the *β*-carotene/linoleic acid assay is based on the discoloration of *β*-carotene caused by radicals released upon the oxidation of linoleic acid in the emulsion. These free radicals can be neutralized in the presence of antioxidants (Benites, 2015). In this study, antioxidant activity of extracts was expressed as percent inhibition relative to the control and measured at various time intervals (Figure 2). Results showed that all *S. japonicum* extracts were able to reduce the coupled oxidation of β-carotene and linoleic acid. The discoloration of β-carotene was greatly slowed down especially in the presence of EaES, EaEB and EaEF. Results indicated that the most active extracts were EaES, EaEB, EaEF, AqES, AqEF and AqEB which displayed an antioxidant activity (AA) greater than 70%. It should be noted that the antioxidant response of the extracts in the β-carotene/linoleic acid model system had a similar pattern as observed in the DPPH assay. Oxidation inhibition of linoleic acid coupled with that of β-carotene appears to be very useful as a mimetic model of lipid peroxidation in biological membranes (Chokki et al., 2020). An extract that inhibits β-carotene bleaching can be described as free radical scavenger, suggesting that the antioxidant activity could be related to free hydroxyl groups of its compounds (Russo, 2018).

Ferrous ion chelating activity

AqEB, AqES and EaES fractions possessed the ability to chelate Fe2+ ions and have potent chelating activity of 53.30 ± 3.42, 66.83 ± 4.01 and 136.93 ± 10.92 µg/mL, respectively; comparable to EDTA as standard (figure 3). The chelating activity is related to the content and nature of the phenolic compounds in the extracts (Arora, 2017). Flavonoids are good chelators of iron, which is one of the mechanisms of their antioxidant activity.

Antibacterial activity

The results obtained for the antibacterial tests of *S. japonicum* extracts were showed in Table 2. Significant and variable antibacterial effects with inhibition zones ranging from 7 to 29.5 mm were obtained on both Gram+ and Gram-pathogenic bacteria, except *C. freundii* and *E. faecalis*. The most active extract was EaES against *E. coli* (29.5 mm), *P. mirabilis* (23 mm), *K. pneumonia* (15.5 mm), *B. cereus* (15.5 mm), *S. typhimurium* (13.5 mm) and *L. monocytogenes* (13 mm). Whereas, *Bacillus cereus* was sensitive to HeEF, EaEB and EaEF with a significant inhibition zone of 20, 13 and 10.5 mm, respectively. Most extracts had an antibacterial activity comparable to the reference antibiotic. These activities were also nearby those of *S. mollis* extracts mentioned by Iram et al. (2013).

Moreover, *C. freundii* and *E. faecalis* were sensitive only to EaEF and AqEF with similar inhibition zones.

Analysis of the data revealed, as for the antioxidant activities, that the fraction having a potent antibacterial was EaES. This could be related to variation of their chemical constituents. Some authors have reported that certain flavonoids can cause the lysis of cytoplasmic membrane or block the synthesis of DNA by the inhibition of topoisomerase (Makarewicz et al., 2021). Other studies have shown that flavonoids such as quercetin and apigenin are involved in the inhibition of D-alanine-D-alanine ligase, thereby disrupting the synthesis of the bacterial wall (Gorniak et al., 2019).

Minimal inhibitor concentration (MIC) and Minimum Bactericidal Concentration (CMB)

The MIC and MBC of the extracts were employed to evaluate their bacteriostatic and bactericidal properties. The MIC was studied on extracts (EaES, EaEB, HeEF and AqEF) with an inhibition zone greater than10 mm against the sensitive bacteria strains. Results in Table 3**,** showed an inhibition of bacterial growth with different sensitivity, where MIC ranging from 0.7 to 86.25 mg/mL and CMB from 5.63 to 431.25 mg/mL. Classification of extracts activity according to their MIC value: active: MIC≤1mg/mL, moderate: MIC = 1-8 mg/mL and weak: MIC = 8-12.5 mg/mL against microorganisms (Katerere et al. 2008). Hence, EaES exhibited a MIC of 0.7 mg/mL against *E. coli* and *B. cereus;* and HeEF gave 0.88 mg/mL on *B. cereus*. These results were comparable to that mentioned by Park et al. (2009). Moderate MIC of 5.62 mg/mL was obtained with EaES on *P. mirabilis*, *L. monocytogenes* and *K. pneumonia*. Where, EaEB gave a MIC of 8.75 mg/mL on *B. cereus*. Furthermore, MBC/MIC ratio was used to estimate antibacterial activity. A ratio ≤ 4 indicate a bactericidal nature of extract and less than 4 is bacteriostatic (Eben Etam et al., 2018; Mogana et al., 2020). Results showed that EaES was bactericidal against five strains: *P. mirabilis*, *B. cereus*, *K. pneumoniae*, *L. monocytogenes and E. coli*; but bacteriostatic on *S. aureus*. Concerning HeEF was bactericidal on *B. cereus;* while, EaEB and AqEF were bacteriostatic against this bacterial strain (Table 3).

Synergistic effect of plant extracts and standard antibiotic

The synergistic effect of plant extracts can be exploited to reduce the dose of the antibiotic and minimize adverse effects. In this study, various and significant antibacterial activities have been obtained by combining the plant extracts with the standard antibiotic (Table 4). The most active fraction was EaES against several bacterial strains. The best synergistic effect (50mm) was obtained on *P. mirabilis*, by combining CRO with this extract. The same combination exhibited synergy against *E. coli* (35mm), *S. typhimurium* (32mm), *L. monocytogenes* (22mm), *P. aeroginosa* (29), *K. pneumonia* (21mm) and *B. cereus* (17mm). These inhibition zones were significantly greater than that of the single antibiotic. However, the fraction EaES combined with Oxa was less effective on *S. aureus*. On the other hand, the fractions EaEB, HeEF and AqEF were also active on *B. cereus* when combined to CRO. Our results are in agreement with studies, which have shown good synergy on the pathogenic germs (Akinbobola et al., 2014).

Table 2. Antibacterial activity of seed (EaES, AqES), stem bark (HeEB, EaEB, AqEB) and fruit pod (HeEF, EaEF, AqEF) extracts from *S. japonicum*; and standard antibiotics (CRO: Ceftriaxone, CTX: Cefotaxime, OXA: oxacillin, CAZ: Ceftazidime). The results were expressed as mean ± SD of triplicate determinations.

Bacterial strains	Inhibition zone (mm)								
	EaES	AqES	HeEF	EaEF	AqEF	HeEB	EaEB	AqEB	A
Pm	23±0.6	_	_	_	_	_	_	_	32 (CRO)
St	13.5±0.7	_	_	_	_	_	_	_	25 (CRO)
Bc	15.5±0.7	7.5±0.7	20±0.7	10.5±0.7	10.5±0.7	8±1.41	13±0.28	_	10 (CRO)
Cf	_	_	_	7.5±0.28	8±1.41	_	_	_	
Kp	15.5±0.7	_	_	_	_	_	_	_	15 (CRO)
Lm	13±1.41	_	_	_	_	_	_	_	17 (CTX)
Sa	9±00	_	_	_	_	_	_	_	27 (OXA)
Pa	10±00	8.5±0.7	_	_	8.5±1.41	_	8±00	8.5±1	20 (CAZ)
Ec	29.5±0.7	_	7±0.7	_	_	_	_	_	29 (CRO)
Ef	_	_	_	8.5±2.12	8±0.7	_	_	_	17 (CRO)

Pm: P. mirabilis, St: *S. typhimurium, Bc: B. cereus, Cf: C. freundii, Kp*: *K. pneumoniae, Lm: L. monocytogenes, Sa: S. aureus, Pa: P. aeroginosa, Ec: E. coli, Ef: E. faecalis*. A: antibiotic. −: indicates no inhibition.

Table 3. MIC and MBC (mg/mL) of seed (EaES), stem bark (EaEB) and fruit pod (HeEF, AqEF) extracts from *S. japonicum*. R=MBC/MIC, MBC/MIC ≤ 4: Bactericidal effect, MBC/MIC> 4: Bacteriostatic effect.

Bacterial strains	EaES			EaEB			HeEF			AqEF		
	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R
Pm	5.62	11.25	2									
Bc	0.7	1.4	2	8.75	43.75	5	0.88	3.52	4	86.25	431.25	5
Kp	5 .62	11.25	2									
L m	5.62	5.62	2									
S a	11.25	67.5	6									
E c	0.7	0.7	1									

Pm P. mirabilis, Bc: B. cereus, Kp: *K. pneumoniae, Lm: L. monocytogenes, Sa: S. aureus, Ec: E. coli*.

Table 4. Antibacterial activity of seed (EaES), stem bark (EaEB) and fruit pod (HeEF, EaEF, AqEF) extracts from *S. japonicum* extracts combined with standard antibiotics (CRO: Ceftriaxone, CTX: Cefotaxime, OXA: oxacillin, CAZ: Ceftazidime).

Bacterial strain	Inhibition zone (mm)					
	EaES+A	HEEF+A	EaEF+A	AqEF+A	EaEB+A	A
P. mirabilis	50 (S)	_	_	_	_	32 (CRO)
S. typhimurium	32 (S)	_	_	_	_	25 (CRO)
B. cereus	17 (S)	25 (S)	11 (I)	13 (S)	17 (S)	10 (CRO)
K. pneumoniae	21 (S)	_	_	_	_	15 (CRO)
L. monocytogenes	22 (S)	_	_	_	_	17 (CTX)
S. aureus	26 (I)	_	_	_	_	27 (OXA)
P. aeroginosa	29 (S)	_	_	_	_	20 (CAZ)
E. coli	35 (S)	_	_	_	_	29 (CRO)

S: synergy, I: indifference. A: antibiotic. CRO: Ceftriaxone, CTX: Cefotaxime, OXA: oxacillin, CAZ: Ceftazidime.

Conclusion

In this study, we have investigated the antioxidant and antibacterial activities of different organs extracts from *S. japonicum*. The tested extracts revealed that the seeds fractions were the most active in scavenging radicals and chelating iron, and have antimicrobial potential against several pathogenic bacteria both gram+ and Gram-. Moreover, the synergistic effects of natural drug from plant origin in association with antibiotics can provide effective therapy against drug resistant bacteria and minimize the toxicity. This plant could be used as a potential source of natural antimicrobial agents and antiradical compounds with beneficial therapeutic effects. Therefore, further investigations need to be carried out to isolate and identify the bioactive compounds present in the plant extracts.

Acknowledgments

This work was supported by the Algerian ministry of higher education and Scientific Research (MERS/DGRSDT). We would like to thank Pr. Chermat Sabah, Department of Pharmacy, Faculty of Medicine, Ferhat Abbas University of Setif, for the identification of the plant material.

Disclosure statement

The authors declare that they have no conflict of interests.

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