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Phytochemical composition, biological potential and enzyme inhibition activity of *Scandix pecten-veneris* L.*

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Abstract: Objective: *Scandix pecten-veneris* L. is a less studied wild edible herb and is considered an extinct plant species in many parts of the world. This study was designed to evaluate its phytochemical composition and biological potential of *S. pecten-veneris* L. Methods: Phytochemicals including alkaloids, flavonoids, polyphenols, and tannins were determined in extracts of *S. pecten-veneris*. Antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH), while reducing power was tested by ferric reducing/antioxidant power (FRAP) assay. Antimicrobial activity against seven bacterial and four fungal strains was evaluated using agar well diffusion assay. Enzymes inhibition study was performed for urease, phosphodiesterase-I, and catalase-II. Results: *S. pecten-veneris* showed moderate antiradical activity and reducing potential of hydroxyl radicals to about 20% of the initial value. The antioxidant activity of various extracts of *S. pecten-veneris* showed a linear correlation with total phenolic contents in the order of water>*n*-butanol>chloroform>ethyl acetate>methanol extracts. *S. pecten-veneris* leaves showed the highest inhibitory activity against *Staphylococcus aureus* while the highest antifungal activity was observed against *Candida albicans*. The plant extract was most potent against urease enzymes but showed moderate activity against phosphodiestrase-I and carbonic anhydrase-II. Conclusions: Our data demonstrate that in addition to its culinary uses, *S. pecten-veneris* has good medicinal potential and hence could be used for treating some specific health ailments.

Key words: *Scandix pecten-veneris* L.; Phytochemical composition; Antioxidant activity; Antimicrobial potential; Enzyme inhibition

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1 Introduction

Traditional plant medicines hold an important place in the treatment of many diseases, especially in developing countries (Kalemba and Kunicka, 2003). Because of the presence of biologically active compounds, plant-based medicines have been recognized as an effective tool for the treatment of a wide range

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of human diseases (Newmann et al., 2003; Bakkali et al., 2008). The increasing resistance of pathogens to antibiotics and their associated side effects make plant-based therapy an attractive area of research. A number of plants have been recognized with multiple therapeutic potential. *Scandix pecten-veneris* L. commonly known as Shepherd's needle, is a member of the Apiaceae or Umbelliferae family (Table 1), and is one of the species used against infectious diseases in traditional medicines. It is characterized by highly divided curly pinnate leaves and tiny white flowers arranged in clusters called umbels (Press and Gibbons, 1993). *S. pecten-veneris* is a dicot, annual scapose herb (Aglaia, 2014). The plant can grow up to 15–50 cm tall, and has long narrow and pointed fruit of 80 mm in length. It inhabits coastal sites, arable fields, and wasteland, and prefers heavy clay soils. The plant is native to Eurasia and present in Europe, reaching west to the UK and north to Denmark (Wildscreen Arkive, 2002), and is also found in some parts of South Asia. *S. pecten-veneris* along with many other species is listed as an extinct or threatened weed species in many parts of the world (Pinke et al., 2011). It was once widespread and abundant, but has declined considerably probably due to the destruction of field margin habitats and the application of chemical herbicides and fertilizers (Berry et al., 2002). Also the seeds of *S. pecten-veneris* cannot stay dormant for long, increasing the risk of local extinction during times of improper habitat management (Wildscreen Arkive, 2002).

Table 1 Scientific classification

Taxa	Name
Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Apiales
Family	Umbelliferae
Genus	*Scandix*
Species	*pecten-veneris* L.
Botanical name	*Scandix pecten-veneris* L.
English name	Shepherds needle, Venus's-comb

From Aglaia (2014)

S. pecten-veneris grows both in cultivated and waste places and prospers in loam, sandy, and heavy clay soils. It grows mainly during autumn and early winter. Seeds of the annual plant tend to germinate in October and early November (Wildscreen Arkive, 2002), although they can also germinate in spring on arable land and flower between May and August (Press and Gibbons, 1993). The leaves of *S. pecten-veneris* represent the main edible part of the plant and are consumed after being boiled and mixed with oil. They are also used in pies (Newman et al., 2003; Algaia, 2014). Young stems of *S. pecten-veneris* are used as a pot-herb or in salads in both raw and cooked forms (Hedrick and Sturtevant, 1972; Tanaka, 1976). The plant has been used as remedy for toothache and other diseases. The important beneficial health effects include a reduction in the risk of chronic inflammatory diseases (Strzelecka et al., 2005). Ethanolic extracts of *S. pecten-veneris* have been observed to decrease tumor necrosis factor α (TNFα) and nitric oxide synthesis in lipopolysaccharide (LPS)-activated monocytes, and significantly reduce the surface expression of VCAM1 on TNFα-stimulated endothelial cells. Additionally, they can decrease LPS or cytokine-stimulated iNOS mRNA levels (Strzelecka et al., 2005).

The consumption of wild plants in the Khyber Pakhtunkhwa regions of Pakistan has been documented for many years. The region is rich in wild flora and *S. pecten-veneris* is found growing there as a wild herb. Previously, we have determined the nutritional composition of *S. pecten-veneris* (Imran et al., 2007), but the in vitro and in vivo biological activity of this species is largely unknown. Therefore, this study was performed to determine the phytochemical composition and biological activity, including antioxidant, antimicrobial, and enzyme inhibition activity of *S. pecten-veneris*.

2 Materials and methods

2.1 Chemicals

The solvents (chloroform, ethyl acetate, methanol, *n*-butanol) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (USA). Iron (III) chloride ($FeCl_3 \cdot 6H_2O$) and sodium acetate ($CH_3COONa \cdot 3H_2O$) were purchased from Merck (Germany). Enzymes including phosphodiesterase-I and carbonic anhydrase-II were purchased from Sigma-Aldrich (USA) and urease from Sigma-Aldrich (Germany).

2.2 Sample collection

S. pecten-veneris leaves were collected from Tarnab Farm Agricultural area, Peshawar, Khyber Pakhtunkhwa of Pakistan during spring (March to April) 2016. The plant was identified at the Botany Department, Peshawar University, Pakistan. Before analysis any visible dirt or insect parts were removed from the plant samples. Fresh and healthy leaves were dried in the shade for at least two weeks, then crushed and stored in the dark.

2.3 Extraction and phytochemical screening

Extraction was carried out by soaking dry powdered leaves (about 2 g) of *S. pecten-veneris* in methanol (30 ml) for 3 d. The extract was filtered, dried, and subjected to phytochemical screening (Ayoola, et al., 2008; Wadood et al., 2013). Results for the phytochemicals screening were expressed as "+" or "–" for the presence or absence of the phytochemicals, respectively.

2.4 Estimation of phytochemicals

The total phenolic content was quantified using the Folin-Ciocalteu method (Ozyigit et al., 2007). Briefly, a mixture containing 0.5 ml of extract in methanol (1 mg/ml), 2.5 ml of Folin-Ciocalteu's reagent (10% (v/v) dissolved in water), and 2.5 ml $NaHCO_3$ (7.5% (75 mg/ml)) was incubated at 45 °C for 45 min. A blank was concomitantly prepared by mixing 0.5 ml methanol, 2.5 ml Folin-Ciocalteu's reagent, and 2.5 ml $NaHCO_3$. Absorbance was measured at 765 nm using spectrophotometer (SP-3000 plus Optima, Japan). Gallic acid (GA) was used as a standard and total phenol was expressed in terms of gallic acid equivalents (mg of GA/g of extract).

Flavonoid content was determined by the aluminum chloride colorimetric method (Lin and Tang, 2007). Briefly, 1 ml each of $AlCl_3$ (2% (0.02 g/ml) solution in methanol) and methanolic plant extract (1 mg/ml) was mixed and incubated for 1 h at room temperature. Absorbance was measured using the spectrophotometer (SP-3000 plus Optima, Japan) at 415 nm. The same procedure was repeated for standard solutions of rutin (RU). Flavonoid content was determined and expressed in terms of rutin equivalents (mg of RU/g of extract).

The total alkaloid content was estimated gravimetrically using aluminum hydroxide precipitation (Gracelin et al., 2013). For alkaloid determination, 1 g of the ground plant material was suspended in 200 ml acetic acid (10% (v/v) in ethanol) for 4 h. The filtrate was concentrated in a water bath to one-fourth of the original volume, followed by the drop wise addition of concentrated ammonium hydroxide until precipitation of the alkaloid was complete. The precipitated alkaloid was washed with dilute ammonium hydroxide, dried, and weighed.

Total tannin was quantified spectrophotometrically (Gracelin et al., 2013). Briefly, 0.5 g of sample was suspended in 50 ml of distilled water and shaken for 1 h. The mixture was filtered into a 50-ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was mixed with 2 ml of 0.1 mol/L $FeCl_3$ in 0.1 mol/L HCl and 0.008 mol/L potassium ferrocyanide. The mixture was incubated for 10 min and its absorbance was measured at 120 nm. This procedure was repeated for tannic acid as a standard. Total tannin content was expressed in terms of tannic acid equivalent (mg of tannic acid/g of extract).

2.5 Antioxidant activity

2.5.1 DPPH radical scavenging activity

The total antioxidant activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH), according to the previously reported procedure (Dorman et al., 2004; Huang et al., 2012). Leaves of *S. pecten-veneris* were subjected to methanolic extraction (3 d) and fractionated using chloroform, ethyl acetate, *n*-butanol, and water. DPPH (2 ml, 0.1 mmol/L in methanol) and plant extract in various solvents (1 ml, 1.0 mg/ml) were mixed thoroughly, and incubated at room temperature for 30 min. The optical densities of the samples were measured (at 517 nm) using the spectrophotometer (SP-3000 plus Optima, Japan). Vitamin C (ascorbic acid) was used as the positive control. The following equation was used for calculation of DPPH radical scavenging potential: DPPH scavenging effect (%)=$(OD_1–OD_0)/OD_0\times$ 100%, where OD_0 is the optical density of the negative control and OD_1 is the optical density of the sample or the positive control.

2.5.2 Ferric reducing power assay

Ferric reducing/antioxidant power (FRAP) assay was carried out using the method reported by Iamsaard et al. (2014). Plant extracts (1 ml, 1 and 3 mg/ml)

were mixed with phosphate buffer (2.5 ml, pH 6.6) and potassium ferricyanide (2.5 ml, 1% (0.01 g/ml)), incubated for 20 min at 50 °C, and centrifuged after the addition of trichloroacetic acid (2.5 ml, 10% (0.1 g/ml)). The upper layer was separated and mixed with ferric chloride (0.5 ml, 0.1% (1 g/L)) and distilled water (2.5 ml). The optical density (at 700 nm) was measured using the spectrophotometer (SP-3000 plus Optima, Japan). Vitamin C was used as the positive control.

2.6 Antibacterial and antifungal assays

The antimicrobial effects of *S. pecten-veneris* were evaluated against 11 strains of microorganisms including bacteria and fungi. Antimicrobial activity was determined by the agar well diffusion method. Seven bacterial species were tested, selected as representatives of both Gram-positive and -negative classes: *Escherichia coli*, *Salmonella typhi*, *Streptococcus pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. Fungal species tested included *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus parasiticus*, and *Candida albicans*. Ten microliters of bacterial and fungal cultures were added to different flasks containing pre-melted and cooled (45 °C) nutrient agar (1.5% (15 g/L) agar, 0.5% (5 g/L) peptone, and 0.3% (3 g/L) beef extract in distilled water) and potato dextrose agar (0.2% (2 g/L) agar, 0.15% (1.5 g/L) peptone, and 0.04% (0.4 g/L) potato starch in distilled water), respectively. The mixtures were then transferred to different culture dishes, solidified, and using sterile steel cork borer, wells of uniform diameter (6 mm) were made on the solidified agar. One hundred microliters of plant extract (5 mg/ml) were added to wells and incubated at 37 °C for 24 h. Standard drugs and dimethyl sulphoxide (DMSO) were used as the positive and negative controls, respectively. Zones of inhibition were measured in mm (Stepanovie et al., 2003).

2.7 Enzyme inhibition

2.7.1 Urease inhibition assay

Urease inhibition activity was determined using the phenol red method (Amin et al., 2013). Briefly, reaction mixtures comprising 55 μl of phosphate buffer (pH 6.8), 25 μl of Jack bean (*Canavalia ensiformis*) urease, 5 μl of plant extract (0.2 mg/ml) in DMSO and 100 mmol/L of urea were incubated at 30 °C for 15 min in 96-well plates followed by the addition of 45 μl of phenol reagent (0.005% (0.05 g/L) sodium nitroprusside and 1% (0.01 g/ml) phenol), and 70 μl of alkali reagent (0.1% (1 g/L) NaOCl and 0.5% (5 g/L) NaOH). Optical density was measured at 630 nm in a microplate reader (Molecular Devices, CA, USA). Thiourea was used as the standard inhibitor. Percent urease inhibition was calculated as follows:

$$\text{inhibition (\%)}=1-(OD_{sample}/OD_{control})\times 100\%, \quad (1)$$

where OD_{sample} is the optical density of the sample and $OD_{control}$ is the optical density of the control.

2.7.2 Phosphodiesterase-I inhibition assay

Phosphodiesterase-I activity (snake venom) was determined as described previously (Rauf et al., 2015). Tris-HCl (33 mmol/L), magnesium acetate (30 mmol/L) buffer (pH 8.8), plant extract and enzyme (0.742 mU/well) were incubated at 37 °C for 30 min followed by the addition of 0.33 mmol/L bis-(*p*-nitropheny1) phosphate. The optical density was measured at 410 nm in a plate reader (Molecular Device, CA, USA). Ethylene diamine tetra acetate (EDTA) was used as the positive control. The percent phosphodiesterase inhibition activity was calculated by Eq. (1).

2.7.3 Carbonic anhydrase-II inhibition assay

Carbonic anhydrase-II (CA-II) inhibition assay was performed as reported previously (Iyer et al., 2006), with slight modification. Briefly, the HEPES-Tris solution (20 mmol/L, 140 μl, pH 7.2–7.9) was mixed with 20 μl of bovine erythrocyte CA-II (0.1 mg/ml in deionized water), 20 μl of plant extract and 4-nitrophenylacetate (4-NPA) at a concentration of 0.8 mmol/L. The mixture was incubated for 15 min and the optical density was monitored at 1 min intervals for 30 min in a plate reader (Molecular Device, CA, USA) at 400 nm. Acetazolamide was used as the positive control, CA-II inhibitor. The percent carbonic anhydrase-II inhibition activity of *S. pecten-veneris* was calculated by Eq. (1).

2.8 Statistical analysis

All experiments were performed in triplicate and results were expressed as mean±standard deviation (SD). Student's *t*-test was used to test differences

between the means and a *P*-value of <0.05 was regarded as statistically significant.

3 Results and discussion

3.1 Phytochemical composition

Phytochemical screening of *S. pecten-veneris* revealed the presence of flavonoids, phenols, tannins, reducing sugars, cardiac glycosides, etc. However, saponins, pholobatanins, or anthraquinones were not detected in plant leaves (Table 2). A quantitative analysis was carried out for alkaloids, flavonoids, phenols, and tannins (Table 3). The methanolic extract was found to contain (48.90±3.93), (98.00±6.78), (48.00±4.45), and (65.00±3.93) mg/g (on dry weight basis) alkaloids, flavonoids, tannins, and phenols, respectively. Flavonoids have been shown to have broad biological activity including antiviral, anti-inflammatory, antibacterial, vasodilatory, antineoplastic, antithrombotic, and antiallergic properties (Miller, 1996). To determine the total phenolic contents, the methanolic extract of *S. pecten-veneris* was further fractionated using various organic solvents including, ethyl acetate, chloroform, *n*-butanol, and water (Table 3). High phenolic content was observed in the water fraction ((100.00±2.15) mg/100 g), while the values for ethyl acetate, chloroform, and *n*-butanol were (10.00±0.72), (8.00±0.40), and (12.00±0.89) mg/g, respectively. However, the values in all cases were lower than those reported by Simopoulos (2004). These differences could be due to geographical location, plants age, or soil composition as these factors have a great impact on the nutrient contents, phytochemical composition, antioxidant activity, and biological potential of plants (Pinos-Rodríguez et al., 2008; Khattak and Rahman, 2015).

Plant phenols have an excellent capability to inhibit enzymes that cause inflammation, act as antioxidants, and modify the prostaglandin pathways thereby protecting platelets from clumping. Nowadays, the search for natural antioxidants is growing for three main reasons: (1) the consumption of fruits and vegetables rich in antioxidants is associated with decreased risks of developing chronic diseases including diabetes, cancer, and cardiovascular disorders; (2) safety concerns regarding the potential harmful effects of the chronic intake of synthetic antioxidants, such as butylhydroxytoluene and butylhydroxyanisole, in beverages and foods; and (3) the public's perception that synthetic antioxidants are more harmful than their natural analogues (Dastmalchi et al., 2007).

Table 2 Phytochemical screening of *Scandix pecten-veneris* L.

No.	Phytochemicals	Inference
1	Flavonoids	+
2	Tannins	+
3	Terpenoids	+
4	Steroids	+
5	Caumarins	+
6	Emodins	+
7	Alkaloids	+
8	Reducing sugar	+
9	Phenols	+
12	Saponins	−
13	Pholobatanins	−
14	Anthraquinones	−

Table 3 Phytochemical composition of *Scandix pecten-veneris* L.

No.	Phytochemicals	Quantity (mg/g dry weight)
1	Alkaloids	48.90±3.93
2	Flavonoids*	98.00±4.78
3	Tannins**	48.00±3.45

* Rutin equivalent (mg of rutin/g of extract); ** Tannic acid equivalent (mg of tannic acid/g of extract). Data are expressed as mean±SD

3.2 Antioxidant activity

Free radicals have been found to be a cause of some major diseases including heart diseases, diabetes, cancer, and aging. Reduction of these radicals by antioxidants is crucial to overcome these health risks. Polyphenolic compounds and flavonoids present in plants act as antioxidants and show remarkable biological potential against these life threatening diseases (Lobo et al., 2010). Both 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and DPPH radicals are used to assess the free radical scavenging abilities of plant extracts and pure compounds. The antioxidant activity of leaf extracts of *S. pecten-veneris* was determined on the basis of their DPPH radical scavenging potential and compared with vitamin E, which was used as a standard. The DPPH radical scavenging activity of different extracts (crude methanolic extract and ethyl acetate, chloroform, *n*-butanol, and water fractions) of *S. pecten-veneris* leaves is given in Fig. 1. All these

extracts were used at a concentration of 1.0 mg/ml. A crude methanolic extract of *S. pecten-veneris* showed 40% scavenging potential against DPPH (Fig. 2). The aqueous fraction showed the highest activity (P<0.001 vs. other fractions) against DPPH, followed by *n*-butanol, chloroform, and ethyl acetate fractions. This means that non-polar antioxidants contribute to the antioxidant activity. Vitamin C which was used as a positive control exhibited the highest DPPH scavenging potential (P<0.01 vs. water fraction and P<0.000 vs. other fractions). The antioxidant potential of *S. pecten-veneris* could be attributed to the synergistic effect of various phytochemicals including steroids, flavonoids, and phenolic compounds. The amounts of various phytochemicals obtained in the current study are lower than those reported by Simopoulos (2004), but are in agreement to those of Pieroni et al. (2002).

The reducing power of an extract or a pure compound represents its ability to act as antioxidant.

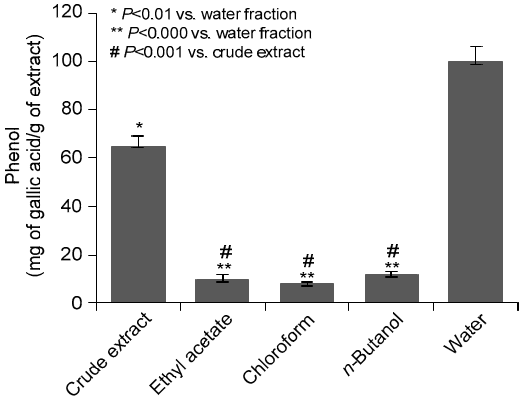

Fig. 1 Total phenol content in various fractions of *Scandix pecten-veneris* extract
Data are expressed as mean±SD

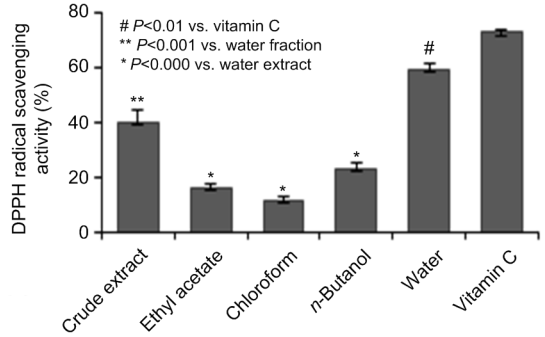

Fig. 2 DPPH radical scavenging activity of different fractions of *Scandix pecten-veneris* extract
Data are expressed as mean±SD

The FRAP assay was used to determine the total antioxidant potential of *S. pecten-veneris*. The FRAP test is considered an accurate indicator of total antioxidant activity (Tezcan et al., 2009). The *S. pecten-veneris* reducing power assay was performed using different extracts at different concentrations (1 and 3 mg/ml) of methanol, *n*-butanol, ethyl acetate, water, and chloroform with vitamin C as a standard (Fig. 3). Among these extracts, the reducing potential of the water fraction was significantly higher than that of other fractions (P<0.000), while the reducing potential of the ethyl acetate fraction was the lowest. Crude methanolic extract was more potent than non-polar fractions in reducing free radicals. The reducing ability of vitamin C was higher than that of the plant extracts. This high reducing power indicates that the compounds present in the extracts are electron donors and can reduce the oxidized intermediates of protein and lipid per oxidation processes, and can serve as primary and secondary antioxidants.

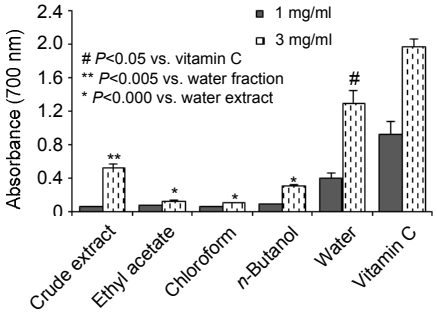

Fig. 3 Reducing power assay of different fractions of *Scandix pecten-veneris* extracts
Data are expressed as mean±SD

3.3 Antibacterial and antifungal activity

The increasing number of infectious diseases poses a major threat to human health globally. The growing resistance of pathogens to antibiotics is exacerbating this problem (Alli et al., 2011). The isolation and synthesis of new compounds with good antimicrobial activity have thus become imperative. The methanolic extract of *S. pecten-veneris* showed variable degrees of antimicrobial activity both for bacteria and fungi. Table 4 shows the inhibitory activity identified in the plant extracts against both Gram-negative and -positive bacterial strains, which are known to cause gastrointestinal, urinary, and respiratory disorders in humans. Streptomycin and

gentamycin were used as standard drugs for comparison. Most of the tested bacteria showed an appreciable sensitivity towards the plant extract. The plant extract showed good activity against *P. vulgaris*, *B. subtilis*, and *S. pneumoniae*, but was most active against *E. coli*, *S. aureus* and *S. typhi*. The increasing order of the size of the zone of inhibition of different bacteria was *P. aeruginosa*<*P. vulgaris*< *B. subtilis*=*S. pneumoniae*<*E. coli*<*S. typhi*<*S. aureus*. *S. aureus* showed the largest zone of inhibition ((22.00±1.17) mm), while *P. aeruginosa* was not sensitive to the *S. pecten-veneris* extract. The antibacterial activity of the plant extract was compared with that of streptomycin and gentamycin as standard antibiotics. The results showed that standard antibiotics have higher activity than the crude extract.

Table 4 Antibacterial activity of methanolic extract of *Scandix pecten-veneris* L.

No.	Bacterial strain	Zone of inhibition (mm)		
		Extract	Streptomycin*	Gentamycin*
1	*E. coli*	18.00±1.02	25.00±1.34	NT
2	*S. typhi*	20.00±1.14	NT	28.00±1.21
3	*S. pneumoniae*	16.00±0.94	35.00±1.18	NT
4	*P. vulgaris*	15.00±0.75	33.00±1.07	NT
5	*P. aeruginosa*	NS	28.00±0.91	NT
6	*S. aureus*	22.00±1.17	NT	28.00±1.32
7	*B. subtilis*	16.00±0.92	NT	26.00±1.19

* Standard drugs. NT: not tested; NS: not sensitive. Data are expressed as mean±SD

The potential antifungal activity was evaluated using four fungal strains as tester microorganisms, selected from among mycotoxin producers, food contaminators, and food spoilage pathogens including *A. flavus*, *A. niger*, *A. parasiticus*, and a clinical oral isolate *C. albicans*. Results of the antifungal potential of *S. pecten-veneris* are given in Table 5. *S. pecten-veneris* showed the highest activity against *C. albicans* with a zone of inhibition of (30.00±1.25) mm and the lowest activity against *A. parasiticus* ((12.00± 1.11) mm). The plant extract produced zones of inhibition with (21.00±2.45) and (23.00±1.78) mm against *A. flavus* and *A. niger*, respectively. Clotrimazole, used as a standard drug against *A. flavus* and *C. albicans* produced (28.00±1.36) and (35.00± 1.22) mm zones of inhibition, respectively, while the standard drug fluconazole showed (29.00±1.02) and (30.00±1.10) mm inhibitory activity against *A. niger* and *A. parasiticus*, respectively. The antifungal property of *S. pecten-veneris* extract was lower than that of the standard drugs, but demonstrated a promising effect on the growth of *C. albicans*. The antimicrobial potential of plant species depends on their chemical composition. The phenolic compounds and flavonoids found in *S. pecten-veneris* could be responsible for its antimicrobial potential against the tested microorganisms.

Table 5 Antifungal activity of methanolic extract of *Scandix pecten-veneris* L.

No.	Fungal strain	Zone of inhibition (mm)		
		Extract	Clotrimazole*	Fluconazole*
1	*A. flavus*	21.00±2.45	28.00±1.36	NT
2	*A. niger*	23.00±1.78	NT	29.00±1.02
3	*A. parasiticus*	12.00±1.11	NT	30.00±1.10
4	*C. albicans*	30.00±1.25	35.00±1.22	NT

* Standard drugs. NT: not tested. Data are expressed as mean±SD

3.4 Enzyme inhibition

Natural products from medicinal plants are widely used in the treatment of many diseases as they inhibit many pathogenic enzymes. Secondary metabolites provide a good pharmacophore template for new drugs. The methanolic extract of *S. pecten-veneris* was assessed for in vitro enzyme inhibitory potential against urease, phosphodiestrase-I, and carbonic anhydrase-II. The extract was found to be significantly more active against urease than standard thiourea but showed low activity against phosphodiestrase-I and carbonic anhydrase-II (Table 6). Phosphodiesterases (PDEs) control cellular concentrations of cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) by hydrolyzing them to 5'-GMP and 5'-AMP, respectively. Inhibitors of PDEs are being used in many pharmacological applications for their vasodilatory, cardiotonic, antidepressant, smooth muscle relaxant, anti-inflammatory, and bronchodilator effects. Many plant metabolites have been shown to be active PDE inhibitors (Rahimia et al., 2010). Mammalian α-carbonic anhydrases have important physiological functions in respiratory homeostasis, pH balance, regulation of intracellular osmotic pressure, and gluconeogenesis. Consequently, a number of carbonic anhydrase isoenzymes are the intended targets of drugs or inhibitors. These inhibitors are commonly used for the treatment of

gastric and duodenal ulcers, hypertension, certain types of cancers, epilepsy, and as antiglaucoma drugs (Winum et al., 2006; Supuran, 2008). Furthermore, carbonic anhydrase isoenzymes present in prokaryotic mammalian pathogens including *Neisseria gonorrhoeae*, *Helicobacter pylori*, and the eukaryotic malarial parasite *Plasmodium falciparum* could also be targeted by these inhibitors. Urease catalyzes the hydrolysis of urea to ammonia, which is important for growth and survival of *H. pylori* in the acidic environment of the stomach and as a result causes various gastrointestinal disorders (Hiyama and Wynder, 1986; Devesa et al., 1998; Shabnam et al., 2015). Many medicinal plants have long been applied as remedies to cure such diseases. *Euphorbia decipiens* (Ahmad et al., 2003) and sulfated polysaccharide of brown seaweed are examples of natural substances reported to have urease inhibition activity (Limuro and Wakabayashi, 2003). The data on enzyme inhibitory activity clearly show the potential of *S. pecten-veneris* as an active inhibitor of urease and hence suggest that it could be effective in the treatment of gastrointestinal disorders. However, further work is required on the isolation and correct identification of the active constituents of the extract.

Table 6 Enzyme inhibitory activity of methanolic extract (0.2 mg/ml) of *Scandix pectin-veneris* L.

Group	Inhibitory activity (%)		
	Urease	Phosphodiestrase-I	Carbonic anhydrase-II
Extract	72.80±5.25	33.11±3.98	44.90±3.32
Standard	98.20±4.72 (Thiourea)	80.10±5.25 (EDTA)	89.00±2.25 (Acetazolamide)

Data are expressed as mean±SD

4 Conclusions

S. pecten-veneris L. showed inhibitory activity against a wide range of Gram-negative and -positive bacteria and fungi, as well as good free radicals scavenging potential. A crude methanolic extract of *S. pecten-veneris* exhibited excellent inhibitory activity against urease, suggesting its possible use in treating gastrointestinal disorders. Data obtained in this study could be useful for determining the detailed chemical composition of *S. pecten-veneris*, as currently little is known about its chemical characteristics. Data on its antioxidant, antimicrobial, and enzyme inhibition effects confirm the medicinal value of this plant and suggest new therapeutic uses for its metabolites or their derivatives. Based on its medicinal potential, *S. pecten-veneris* could be of great importance to pharmaceutical industries if it is properly exploited.

Compliance with ethics guidelines

Abdul WAHAB, Syed Aleem JAN, Abdur RAUF, Zia ur REHMAN, Zahid KHAN, Aftab AHMED, Fatima SYED, Sher Zaman SAFI, Hamayun KHAN, and Muhammad IMRAN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：*Scandix pecten-veneris* L.的植物素、生物学潜力及酶抑制活性研究

目　的：本研究旨在测定 *Scandix pecten-veneris* L.的植物素和评价其生物学潜力。

方　法：测定 *S. pecten-veneris* 提取物中植物素的含量，包括生物碱、黄酮、多酚和单宁。使用 2,2-二苯基-1-苦肼基（DPPH）测定抗氧化活性；同时通过铁还原/抗氧化能力（FRAP）测定还原能力；使用琼脂扩散测定法评价对七种细菌和四种真菌菌株的抗微生物活性。同时，对脲酶、磷酸二酯酶-I 和过氧化氢酶-II 进行酶抑制研究。

结　论：沙门氏菌显示出适度的抗自由基活性；羟基自由基的潜能降至初始值的 20%左右。*S. pecten-veneris* 多种提取物的抗氧化活性与总酚含量呈线性相关。*S. pecten-veneris* 叶对金黄色葡萄球菌表现出最高的抑制活性；对白色念珠菌表现出到最高的抗真菌活性。植物提取物对脲酶的活性最有效；对磷酸二酯酶-Ⅰ和碳酸酐酶-II 显示出中等活性。结果表明，*S. pecten-veneris* 具有良好的药用潜力，可用于治疗一些特定的疾病。

关键词：*Scandix pecten-veneris* L.；植物素；抗氧化活性；抑菌能力；酶抑制