Phytochemical Analysis, Antioxidant and Antimicrobial Screening of Seriphidium Oliverianum Plant Extracts

Ali Abbas1, Syed Ali Raza Naqvi1, Muhammad Hidayat Rasool2, Asma Noureen3, Muhammad Samee Mubarik4, and Rasool Baksh Tareen5

Abstract
The aim of this study was to investigate the phytochemicals using reverse-phase high pressure liquid chromatography (RP-HPLC), antioxidant, antifungal and antibacterial activities of Seriphidium oliverianum stem extracts. The extraction was carried out by conventional shaking process (CSP) and ultrasonic assisted process (UAP). The highest total phenolic contents (97.85 ± 0.735 mg gallic acid equivalent (GAE)/g sample) and flavonoid contents (188.15 ± 0.53 mg catechin equivalent (CE)/g sample) were found in methanol extract obtained by CSP. Antioxidant activity was investigated using DPPH scavenging assay and reducing power assay. Methanol extract using UAP showed the highest DPPH scavenging activity (79.95% ± 1.80%) followed by methanol and butanol extracts obtained through CSP. Moreover, methanol extracts using CSP showed highest reducing activity (1.032 ± 0.0205 absorbance). In-vitro antimicrobial activity was studied using most common infection causing fungal and bacterial strains. Antifungal activity of methanol extract using CSP showed the highest zone of inhibition (10.5 mm) against F. avenaceum fungal strain, while aqueous extracts obtained through showed the highest antibacterial activity (22 ± 1.32 mm zone of inhibition) against S. aureus. The results showed that the methanol stem extract of S. oliverianum is a valued candidate for further screening and could be processed for in-vivo infection induced animal trials.

Keywords
Seriphidium oliverianum, solvent extraction, RP-HPLC, polyphenols, antioxidants, antimicrobial activity

Introduction
Baluchistan is the largest province of Pakistan in term of land area which located southwest of country with coordinates 27.7°N 65.7°E. Its land comprises of variety of variations in land structure and climates. It mainly consists of vast deserts, forests and mountains. Its big area also touches the sea with distinction of Gwadar Port. The environment in all parts of the province shows great variation—hot to quite cool and pleasant. The variation in weather makes it rich in medicinal floras which are known to whole world for its therapeutic potentials. S. oliverianum is medicinally least explored specie which belongs to the Seriphidium genus and Asteraceae family, and is native to Baluchistan region. The plant mainly grows in sandy clay (40-60 cm tall) having suffrutescent shrublet and 5-7 greenish-yellow florets (flower period August to September). The plant also grows in neighbor to Baluchistan such as Iran, Afghanistan and Central Asia (Turkmenia).

Various species of genus Seriphidium are well known for its folk medicine utilization over the globe; however, S. oliverianum first gained the fame due to α-thujone, β-thujone, 1,8-cineole and β-caryophyllene compounds which were identified in its essential oil.1 The leave extract of the plants also a source of pretty good antioxidants such

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1 Department of Chemistry, Government College University, Faisalabad, Pakistan
2 Department of Microbiology, Government College University, Faisalabad, Pakistan
3 Department of Zoology, Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Defense Road Campus, Lahore, Pakistan
4 Department of Zoology, Government College University, Faisalabad, Pakistan
5 Department of Botany, University of Balochistan, Quetta, Pakistan

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Corresponding Author: Syed Ali Raza Naqvi, Department of Chemistry, Government College University, Faisalabad 3800, Pakistan. Email: drarnaqvi@gmail.com; draliraza@gcuf.edu.pk
as gallic acid, kaempferol 3-O-β-D-glucopyranoside and 1,2,4,6-O-tetra-galloyl-β-D-glucopyranoside. Nusrat et al (2014) reported 11 phytochemicals isolated from *S. oliverianum* of medicinal interest which had already been isolated from other sources as well. In another report the compounds isolated from *S. oliverianum* leave extract; 3,6-Dimethylquercetagetin-7-O-β-D-glucoside and 5,3',4'-trihydroxy-6,7-dimethoxyflavone showed mild antioxidant (IC_{50} = 130 µg/mL), and urease inhibition potential (IC_{50} = 129 µg/mL), respectively.

Antioxidant, anti-microbial and number of other biological activities are known as phenolics and all bear at least 1 aromatic ring occupied with 1 or more hydroxyl group/s. These compounds are the custodian of variety of biological activities such as antioxidant, antibacterial, anticancer, etc. and could be termed as index of phytomedicine. The alkaloids (piperine, berberine, dictamine, kokusagine, masculine, reserpine, sanguinarine, tomatidine, chanoclavine), organosulfur compounds (allicin, ajoene, isothiocyanates, sulforaphane, phenethyl isothiocyanate, berteroin), 

The aim of this work was to analyze the phenolic and flavonoid compounds, antioxidant and antimicrobial potential of *S. oliverianum* stem extracts using CSP and UAP. To our best knowledge, few reports have been published on leave extracts, so there is a need to explore the medicinal potential of extracts of other parts of *S. oliverianum*.

**Material and Methods**

**Material**

*S. oliverianum* was initially collected from deserts of District Quetta, Baluchistan in September 2013, and identified by Prof. Dr. Rasool Bakhsh Tareen, Plant Taxonomist, Department of Botany, University of Baluchistan, Quetta and assigned voucher specimen SO-RBT-06 to save in university herbarium. All the plant parts were separated, washed with distilled water to remove dust particles, and placed in shaded area under aerated condition for 7 days at room temperature. The dried stem part of the plant was pulverized into fine powder using domestic grinder and saved in air-tight bags for further solvent extraction. All the solvents and chemicals that were used in this research work were of analytical grade and obtained from Sigma-Aldrich (Germany) with exception of methanol and butanol that was obtained from Alfa-Acer. The sonicater (VC 130, Sonics & materials, Inc, Newtown, CT) was purchased from USA. The HPLC system was of Perkin-Elmer (USA).

**Preparation of Extracts**

Different solvents such as methanol, butanol and water were used to extract phytochemicals from the stems of *S. oliverianum* using CSP and UAP. Briefly, CSP was carried out by mixing the dried sample powder (50 g) into 500 mL of solvent followed by subjecting the flask on orbital shaker for 8 h at 200 rpm and room temperature. While the extraction through UAP was carried out by mixing 10 g of dried powder into 100 mL of solvent followed by sonication procedure for 15 min at 50% amplitudes, 20 kHz frequency and 25 mm probe at room temperature using sonicater. Following the extraction process, the mixture was filtered through Whatman filter paper 1, filtrate was evaporated under reduced pressure using Heidolph rotary evaporator and finally the concentrated extract was stored at −4°C until further analysis. Percent yield of all extracts were calculated using following formula:

\[
\text{Yield (\%)} = \left(\frac{\text{Weights of solvent free extract (g) \times 100}}{\text{Dried extract weight}}\right)
\]

For every sample triplicates flasks were used for extraction and results were expressed as mean ± standard deviation (SD) (n = 3).

**Determination of Total Phenolic Contents**

Total phenolic contents (TPC) were determined using Folin-Ciocalteu method. Briefly, 1 mL of extract sample (15-120 mg/mL) was used for standard calibration curve.

**Determination of Total Flavonoid Contents**

Total flavonoid contents (TFC) were determined using aluminum chloride colorimetric method. Briefly, 0.25 mL of extract (31.25-250 mg/mL) was added into 0.75 mL distilled water, followed by the addition of 0.15 mL of sodium nitrite solution (5%). After 5 min incubation period, 0.3 mL aluminum chloride (10%) was added and the mixture was shaken gently. The mixture was then incubated at room temperature for 90 min. The absorbance was recorded at 765 nm. Gallic acid (15-120 mg/mL) was used for standard calibration curve. Results were expressed as gallic acid equivalents (GAE)/mg dry weight (DW) of extract.

**HPLC Analysis of Phenolic Acids**

Sample preparation. A method reported by Ying et al, (2009) with slight modification was followed to prepare sample.
Briefly, 0.5 g of the dried and powdered stem plant was taken in a conical flask with lid along with a 0.5 mL mixture of standard phenolics, then extraction was performed with 50 mL aqueous mixture of methanol (50% V/V) in an ultrasonic bath for 30 min. The mixture was subjected to centrifugation at 3000 rpm for 5 min at 4ºC. The supernatant was then filtered with a 0.45 μm membrane filter. An aliquot of 20 μL filtrate was injected into HPLC system using micro syringe.

**HPLC system and conditions.** HPLC analysis was performed using Perkin Elmer series 200 HPLC system incorporated with UV/Visible detector. The system was equipped with reverse phase HPLC analytical C-18 column (4.6 × 250 mm, 5μm stationary phase particle size), binary LC pump system and temperature control module. The protocol reported by Wen et al, (2005) with slight modification was followed. The binary solvent mobile phase system for gradient elution was chosen. Water was set as Solvent A, while methanol was as solvent B. Both solvents were acidified with 0.02% trifluoroacetic acid (TFA). The gradient elution was carried out as follow: 0-3 min, 25% B; 3-7 min, 25%-30% B; 7-12 min, 30%-50% B; 12-15 min, 50% B; 15-18 min, 50%-80% B; 18-22 min, 80% B; 22-25 min, 80%-25% B. The flow rate 1.0 mL/min was adjusted at 25ºC column temperature. The detection wavelength 254 nm was selected.

**Determination of Antioxidant Activity**

Antioxidant activity of *S. oliverianum* stem extracts in methanol, butanol and aqueous medium was studied using DPPH scavenging and reducing power assay.

**DPPH free radical scavenging assay.** DPPH scavenging activity of plant extract was calculated by recording the potential of extracted sample to reduce DPPH into DPPH-H (a colorless compound). The antioxidant potential of plant extract was measured by DPPH scavenging assay with mild modification. Briefly, 0.1 mL of plant extract (62.5-500 mg/mL) was added in 2 mL of DPPH solution (0.1 mM) in methanol and the mixture was allowed to stand for 1 h in dark at room temperature. The absorbance was measured at 517 nm. Free radical scavenging activity of plant extract was calculated by using following formula:

\[
\% \text{Scavenging of DPPH} = \frac{A_{\text{Initial Absorbance}} - A_{\text{Final Absorbance}}}{A_{\text{Initial Absorbance}}} \times 100
\]

where the initial and final absorbance are the absorbance values of DPPH at zero time and after 1 h of plant extract addition.

**Reducing power assay.** The reducing power activity of plant extract measured following pre-reported method. Briefly, 1 mL of extract sample (125-1000 μg/mL) mixed with 1 mL of 200 mM sodium phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide solution. The mixture was shaken gently and incubated at 50ºC for 20 min. After incubation period, 1 mL of 10% trichloroacetic acid solution was added followed by centrifugation for 10 min at 3000 rpm. The mixture was converted into supernatant and solid pellet; 2.5 mL supernatant was pipet-out and mixed with 2.5 mL of distilled water followed by the addition of 0.5 mL of 1% aqueous ferric chloride solution and shaken gently. The absorbance of solution was recorded at 700 nm. A typical blank solution contained the same solution mixture without plant extract taken as control while ascorbic acid was used as a reference standard.

**Determination of Antimicrobial Activity**

Anti-microbial activity of different solvent extracts of *S. oliverianum* stems were tested against infection causing fungal and bacterial strains.

**Antifungal activity.** MTCC 1344 strain of *Aspergillus niger* (A. niger), ATCC 60644 strain of *Fusarium avenaceum* (F. avenaceum) and MNCC1206 strain of *Fusarium brachy-gibbosum* (F. brachygibbosum) were used to investigate the anti-fungal activities of plant extracts. The anti-fungal activities of different extracted samples of *S. oliverianum* stems were performed by using well diffusion susceptibility method. Briefly, sterilized nutrient agar medium plates were seeded with 18-24 h old cultures of microbial inocula (standard inoculum of 1-2 × 10⁷ CFU mL⁻¹, 0.5 McFarland standard). Six wells (8 mm diameter) were cut into the solidified agar media with sterilized cork borer followed by the addition of an aliquot of 40 μL (4mg/mL) of extract in triplicate wells. Gentamycin and DMSO of equal volume and concentration were also inoculated into triplicate wells as positive and negative control, respectively. Then inoculated plates were incubated for 24 h at 37ºC. After completion of incubation period, measured the zone of inhibition in mm.

**Antibacterial potential.** ATCC 25923 strain of *Staphylococcus aureus* (S. aureus), ATCC 25922 strain of *Escherichia coli* (E. coli), ATCC 15380 strain of *Klebsisella pneumonia* (K. pneumonia), ATCC 27853 strain of *Pseudomonas aerugi-nosa* (P. aeruginosa), ACTT 17978 strain of *Acinetobacto r baumannii* (A. baumannii), and SSFP(4 s) strain of *Salmonella typhi* (S. typhi) were obtained from clinical setup. All bacterial strains were cultured at 37ºC for 24 h in Mueller Hinton agar (MHA) nutrient. The antibacterial activity of extracts was then carried out using Agar well diffusion method. Sterile MHA was used as a media, for preparation of petri plates. The test strains were swabbed on the surface of solidified media and kept for drying at room temperature for 10 min, after that 5 wells (6 mm diameter) were engraved with sterilized cork borer on the surface of solidified petri plate. Four different concentrations of plant extract (0.5-4 mg/mL) were tested with 3 replications. An aliquot of 40 μL of plant extract was inoculum in each well, and ciprofloxacin (5μg/disk) was used as positive control. After 12-15 min of diffusion time at room temperature, the inoculated plates were incubated at 37ºC for 48 h. At the end of incubation period the antibacterial activity was determined by measuring the zone of inhibition in millimeter (mm) unit of length. The diameter of the inhibition zone was measured in 3 directions and mean value was tabulated.
Values are presented as mean ± SEM and scrutinized by 2-way ANOVA, followed by least significant difference (LSD) test using SPSS software and graph pad prism. The results of all extracts compared with standards. *P value > 0.05 is considered as non-significant (ns), *P value < 0.05 as significant (*) and **P value < 0.001 as highly-significant (**).

Results and Discussion

Extraction Yield

The extraction of phytochemicals from \textit{S. oliverianum} stems in organic and aqueous medium showed good yield. Methanol, butanol and aqueous extracts showed 15.55, 10.16 and 52.30\% yield of dry sample using CSP, respectively—while with UAP, it was recorded 28.48, 7.56 and 42\%, respectively. It was reported that most of the sandy-clay plants of Baluchistan origin show promising biological activities but poor extract yield.\(^2\) Butanol, due to its more lipophilic behavior as compared to methanol and water, showed weak potential to extract phytochemicals; however good yield in aqueous medium might be due to the fact that most of the phytochemicals bear electronegative functional groups which make the compound hydrophilic in nature.

Total Phenolic and Flavonoid Content

The results showed that methanol extract carry good amount of TPC and TFC followed by extraction in butanol using CSP; while UAP assisted significantly to extract TPC and TFC in aqueous medium. The highest TPC ($97.85 \pm 0.735$ mg (GAE)/g sample) and TFC ($188.15 \pm 0.53$ mg (CE)/g sample) were found in methanol extract using CSP while lowest TPC ($42.56 \pm 0.775$ mg (GAE)/g sample) was recorded in aqueous extract as shown in Figure 1; while $142.11 \pm 0.89$ mg (CE)/g sample TFC was recorded in aqueous medium as shown in Figure 2. UAP, however assisted the aqueous medium to improve the extraction of TPC and TFC which increased to $64.39 \pm 0.895$ mg (GAE)/g sample and $219.05 \pm 1.58$ mg (CE)/g sample, respectively as compared to CSP. The findings are in good agreement with the report published previously.\(^3\) It was reported that multiple hydroxyl functional group present in phenolics and flavonoids are responsible of their biological and antioxidant activities. Molluscicidal, anthelmintic, antihepatoxic, anti-inflammatory, anti-diarrheal, antiulcer, vasodilatory action, antiallergic and antiviral activities are in the profile of these compounds. They also play role in soil nitrogen mineralization.\(^4\) Other unique action of these compounds that has been reported are; inhibition of human immunodeficiency viral replication (HIV), Glucosyl-transferases of \textit{Streptococcus mutans}, ascorbate autoxidation, human simplex virus (HSV), tumor proliferation, and xanthine/monoamine oxidases.\(^5\)

HPLC Analysis

RP-HPLC with C18 columns is the most popular technique for the analysis of polyphenols of different foods and plant extracts. A typical HPLC chromatogram of phenolic constituents of \textit{S. oliverianum} stem extract is shown in Figure 3. The amount of phenolic acids detected in the analyzed sample is shown in Table 1. Four phenolic acids; p-coumeric acid, sinapic acid, ferulic acid and caffeic acid were found in good amount while quercetin was detected in least quantity. It was reported that the ferulic acid, p-coumeric acid sinapic acid are actively involved in antimicrobial, antiviral, anti-mutagenesis, anti-cancer and diabetes mitigation, anti-inflammatory, anti-neurodegeneration activities.\(^6,7\) Caffeic acid play good role in therapy of Alzheimer’s Brain disease.\(^8\) Other detected phenolics has also been reported for their promising biological activities.\(^9,10\) Presence of these phenolic acids in \textit{S. oliverianum} stem extract reflects its possible utilization in phytomedicines and food preservation processes.
Analysis of DPPH® Scavenging Activity

DPPH® scavenging assay is known to natural product researchers a most valued procedure to assess the antioxidant activity of the plant extracts. DPPH® (2,2-diphenyl-1-picrylhydrazyl) is dark purple colored stable free radical that converts into colorless 2,2-diphenyl-1-picrylhydrazine (DPPH-H) by accepting a hydrogen atom from phenolic compound. The quenching intensity of the DPPH® directly relates the concentration of phenolic. DPPH® scavenging potential of S. oliverianum stem extracts is shown in Figure 4. All of the assessed stem sample extracts revealed a gradual fading in purple-colored radical DPPH® into the yellow-colored DPPH-H by increasing the extract concentration. Methanol extract using UAP showed highest scavenging ability (79.95 ± 1.80%) followed by methanol and butanol extracts using CSP method, 76.66 ± 1.52 and 75.83 ± 1.60% respectively. Butylated hydroxyl toluene (BHT), which was used as standard DPPH® scavenging molecule showed 91.59 ± 1.52% scavenging activity. Phenolics and flavonoids, among the plant constituents are considered strong free radical scavenging compounds—hence can be termed as the index of free radical scavenging activity.33
Reducing Power Assay

The chemistry of reducing power assay based on the reduction of potassium ferricyanide (Fe$^{3+}$) to form potassium ferrocyanide (Fe$^{2+}$) by components of plant extracts, followed by reacting with ferric chloride (FeCl$_3$) which resulted in the formation of ferric–ferrous complex that has an absorption maximum at 700 nm. The concentration of ferric–ferrous complex formation depends upon the potassium ferrocyanide formation. It was reported as concentration dependent activity, which increases with the increase of natural product contents especially the polyphenolic compounds and flavonoids. Figure 5 shows the dose-dependent reducing power of $S.$ oliverianum stem extract. The highest absorbance was recorded (1.032 ± 0.0205) with 1 mg/mL methanol extract obtained using CSP. Moreover, as compared to other extracts obtained through UAP method methanol extract also showed highest absorbance 0.660 ± 0.0769. Previously reported studies describe that the free radical scavenging activity of plant extracts is correlated with their TPC, and our results are in agreement with their findings. The reducing potential of standard antioxidant, ascorbic acid showed comparatively low absorbance value (0.780 ± 0.110) at similar concentration level.

Antifungal Study

Methanol, butanol and aqueous extracts of $S.$ oliverianum stems, obtained by CSP and UAP, were also evaluated for their anti-fungal activity. The results obtained against the tested microorganisms are shown in Table 2. Among the UAP, the aqueous extract was the most potent which showed the 9.5 ± 0.25 mm ZOI against $A.$ niger while among the CSP process, the methanol and butanol extracts showed 10.5 ± 0.11 and 10 ± 0.14 mm ZOI against $F.$ avenaceum and $F.$ brachygibbosum, respectively appeared most potent. The comparison of anti-fungal activities between the 2 extraction procedures, indicated CSP process assisted more, than UAP process in the
Aqueous CSP 4 8.5 ± 0.5 8.5 ± 0.5 9 ± 0.5
Methanol 4 9.5 ± 0.5 10.5 ± 0.5 10 ± 0.5
Butanol 4 9.5 ± 0.5 10 ± 0.5 10 ± 0.5
Aqueous UAP 4 9.5 ± 0.5 8.5 ± 1 8.5 ± 1
Methanol 4 8.5 ± 0.5 10. ± 0.5 9.5 ± 0.5
Butanol 4 8 ± 0.5 Negative 8 ± 1
Gentamycin (standard drug) 5μg/disk 15 ± 1 15 ± 1 17.5 ± 0.32

Table 3. Antibacterial Activities of Stem Extracts of *S. Oliverianum* Plant Against Different Bacterial Strains.

| Extraction solvent | Extraction technique | Conc. mg/mL | Zone of inhibition value (mm) |
|--------------------|----------------------|-------------|-------------------------------|
|                    |                      |             | *S. aureus* | *P. aeruginosa* | *E. coli* | *K. pneumonia* | *A. baumannii* | *S. typhi* |
| Methanol           | CSP                  | 0.5         | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 1           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 2           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 4           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
| Butanol            | CSP                  | 0.5         | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 1           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 2           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 4           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
| Water              | CSP                  | 0.5         | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 1           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 2           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 4           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
| Methanol           | UAP                  | 0.5         | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 1           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 2           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 4           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
| Butanol            | UAP                  | 0.5         | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 1           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 2           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 4           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
| Water              | UAP                  | 0.5         | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 1           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 2           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
|                    |                      | 4           | 7 ± 0.57 | 9 ± 1.00 | 13 ± 0.14 |
| Ciprofloxacin      | standard drug        | 5μg         | 25 ± 0.15 | 25 ± 0.15 | 11 ± 0.15 |

Values are expressed as mean ± standard deviation.

Antibacterial Activity

Antibacterial activity was tested against 1 Gram positive (*S. aureus*) and 5 Gram negative bacteria (*P. aeruginosa, E. coli, K. pneumonia, A. baumannii & S. typhi*) using agar well extraction of anti-fungal components. The results of all extracts were also compared with standard anti-fungal agent, gentamycin which showed 17.5 ± 1.675, 15.2 ± 1.13 and 14.9 ± 1.18 mm ZOI against *F. brachygibbosum, A. niger* and *F. avenaceum*, respectively. It was reported that some tannins, flavonoids, saponins, steroids, alkaloids mainly and α-bisabolol, a natural monomeric sesquiterpene alcohol to some extent, are responsible for anti-fungal activity. This indicates the *S. oliverianum* stems possess good amount of these compounds as well and could be tested for further evaluation.
diffusion method. Antibacterial activity of all extracts is shown in Table 3. The results showed that all the extract showed good dose-dependent antibacterial activity. The highest growth inhibition potential attributed to 4 mg/mL extract sample. At this concentration in case of CSP; the aqueous extract was the most effective extract which showed the highest 22 ± 1.32 mm ZOI against S. aureus bacteria followed by butanol and methanol extracts (21 ± 1.04 and 18.0 ± 0.28 mm, respectively) against P. aeruginosa—while in case of UAP, butanol extract showed 20.5 ± 0.28 and 19 ± 0.100 mm ZOI against P. aeruginosa and A. baumannii, respectively followed by methanol extract which showed 18 ± 1.32 mm ZOI against P. aeruginosa. Moreover, K. pneumonia, A. baumannii and S. typhi in general, showed resistance to almost all extracts. Butanol and aqueous UAP extracts, however faced strong resistance from S. typhi with zero growth inhibition results. It is worth understanding that all these bacteria are referred to as food poising and food borne diseases; which need to address carefully. Most of the published food poisoning reports and bacterial infections indicated the involvement of members of Gram negative bacteria, especially P. aeruginosa, E. coli, K. pneumoniae, A. baumannii & S. typhi. The Gram negative bacteria also offer more resistance to antibacterial agents as compared to the Gram positive bacterial strains such as S. typhi is one that cause severe food borne illness known as typhoid fever and is treated with a set of antibiotics instead of single antibiotic. The results of the study were compared with the ciprofloxacin taken as standard antibacterial agent, it showed strong growth inhibition of P. aeruginosa with 25.5 ± 1.21 mm ZOI followed by 20.2 ± 1.10 mm ZOI against K. pneumonia, however some results were missed due to COVID-19 epidemic lockdown.

**Declaration of Conflicting Interests**
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**ORCID iD**
Syed Ali Raza Naqvi 🔗https://orcid.org/0000-0002-2172-9066

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**Conclusion and Future Perspective**
It is appealing that methanol, butanol and aqueous S. oliverianum stem extracts showed considerable amount of ferulic acid, p-coumeric acid sinapic acid as detected by RP-HPLC which are indicator that extract of stem could be refined as functional food and phytomedicine. The results of antioxidant such as DPPH scavenging activity and 1.032 ± 0.0205 (absorbance) reducing activity are also promising phytomedicine indicators. Further, 10.5 mm ZOI using F. avenaceum fungal strain and 22 ± 1.32 mm ZOI against S. aureus reflect plant’s medicinal impact. In conclusion, S. oliverianum stem extracts could be subjected for further investigation using preclinical in-vitro and in-vivo models.

**Authors’ Note**
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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