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RESEARCH ARTICLE
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IN VITRO EVALUATION OF *SESUVIUM SESUVIOIDES* (FENZL) VERDC. (AIZOACEAE) OF CHOLISTAN DESERT FOR ANTI-OXIDANT, ANTIMICROBIAL AND CYTOTOXIC POTENTIAL

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Abstract

The aim of current study was to explore the antioxidant, antimicrobial and cytotoxic potential of different extracts (*Sesuvium sesuvioides* ethanol (SS E), *Sesuvium sesuvioides* n-hexane (SS nh, *Sesuvium sesuvioides* distilled water (SS DW). Antioxidant activity of extracts of selected plant were determined by using 2, 2-diphenyl-1-picrylhydrazyl radical scavenging assay as designated by researchers. Folin-Ciocaletu reagent method was used to determine total phenolic contents (TPC). The total flavonoid content (TFC) of the extracts were determined by aluminum chloride complex forming assay. The agar well diffusion method was used, against gram positive and negative bacterial strains. The hemolytic activity was determined by Spectro-photometric method for different extracts against red blood cells and PBS were be used as negative control and Triton-X-100 as positive control. The *Sesuvium sesuvioides* ethanol extract showed maximum antioxidant (radical scavenging) effects 66.667% with IC_{50} value (81.48±1.22) µg/mL which was significant ($P<0.01$). Maximum effect of TPC (mg GAE/g) was exhibited by n-hexane extract 172.4±1.22 mg GAE/g. Similarly, the n-hexane extract showed maximum TFC activity 21.428±2.02mg QE/g. Among three extracts of *Sesuvium sesuvioides*, maximum zone of inhibition against *E. coli* and *B. subtilis* was shown by distilled water extract [(18±0.333) mm], [(19±0.577) mm] respectively. n-hexane extract showed higher hemolytic activity than other extracts (32.525%). Thus, results of study showed that extract of Sesuvium sesuvioides exhibit antimicrobial, cytotoxic and antioxidant potential.

Keywords: Antimicrobial activity, cytotoxic potential, plant extract, Anti-oxidant activity

Introduction

Cholistan desert regionally renowned as Rohi desert that encircles round about 30 km from Bahawalpur, Punjab, Pakistan and making territory of 26,000 km^2 which is wide source of medicinal plants. Its length is 480 km and 32-192 km varying breadth (Ali, Chaudhry, & Farooq, 2009). Cholistan desert is remarkably based on wild area of its own type with shortage of indigenous flora, carrying only 128 species associating to 32 families(Raza, Younas, & Schlecht, 2014). The distinctive medicinal knowledge of plants is not popular, but constrained to the regional herbal consultants called '*hakims*'. They suggest treatments of diseases (either human oriented or plant oriented) by giving herbal extracts, granules, fresh, macerated or dried (Ahmad, Alam, Wariss, Anjum, & Mukhtar, 2014). Many reports are accessible on the anthelmintic, anti-inflammatory, antiviral, antifungal, anti-molluscal, antibacterial properties of plants but the Cholistan desert have not been exposed to scientific analysis for their effectiveness and well-being (Hameed et al., 2011).

The flora of Cholistan desert of Pakistan consists several number of Halophytes (salt-tolerant plants) which is distributed almost in all region of Bahawalpur. These plants are succulent and no ability of secreting salts. Halophytes plants consists of biologically active compounds possessing potent pharmacological effects. (Fatima et al., 2019). Therefore, the halophytes plants are a good source of food products, chemicals, medicine, forage and for the production of biomass for renewable energy (Fatima et al., 2019). Some species of halophyte are used as food, vegetable or salad such as *Haloxylon salicornicum, Atriplex balimus, Chenopodium album, Salicornia europaea, Sesuvium portulacastrum, Portulaca oleracea* and *Suaeda maritime* (El Shaer & Attia-Ismail, 2015). *Suaeda fruticose, Alhagi maurorum, kochia scoparia, Salsola imbricata,* are used as food (Joshi, Kanthaliya, & Arora, 2018). *Chenopodium glaucum and Haloxylon stocksii* are source of edible oil (Joshi et al., 2018). Halophytes such as *Achyranthes aspera, Solanum surattense, Citrullus colocynthis Sesuvium sesuvioides, Capparis decidua* have great medicinal importance; their various species have been used in different diseases such as cold, flu, cough, bronchitis and asthma. (Rafay, Ghaffar, Abid, Malik, & Madnee, 2021).

Sesuvium sesuvioides (Fenzl) Verdc. Is native to Cholistan desert and belongs to Aizoaceae family. Regionally it is called "Lunio" and used as herbal medicine. Traditionally it is used in gouty arthritis by conventional healers. Its stem and preferably roots, when mixed with water after stamped, are used for hemorrhage, chicken pox, smallpox, measles and nose bleeding. It is also used in cough, flu and cold. It is mixed with pot herbs and boiled to make it soft and tasty. It is used as fodder for goats and pigs. *S. sesuvioides* also used in the treatment of thyroid dysfunction, inflammation, fever, ulcer and diabetes by local practitioners in the desert areas of Cholistan. Recently anti-inflammatory, analgesic and antipyretic potential of *Sesuvioides* have been reported scientifically (Sajid-Ur-Rehman et al., 2021). No extensive research has been done on this plant. Therefore, the objectives of the present study were to evaluate the antimicrobial, antioxidant and cytotoxic activity of different extract of leaves of *Sesuvium sesuvioides*.

Material and Method

Chemicals and Reagents

Ethanol (Sigma-Aldrich, Germany), Whattman No 1 filter paper (Sigma-Aldrich, Germany), Nutrient Agar (Oxoid, Hamsphire, UK), Sodium Hydroxide (NaOH) (Merck, Germany), O.45µm filter paper (Biotech, Germany), Catechin (Fluka, Sigma-Aldrich), Sodium Nitrite (Germany appliChem), Ferric chloride (FeCl3)(BDH Laboratory Supplies, England), Aluminum chloride (AlCl3) (Fluka chemic Switzerland), Triton-X (Germany, appliChem), Sodium Acetate (Sigma-Aldrich, Germany), Nutrient broth (LAB M, Limited, UK), Sodium Carbonate (Germany, applic hem), DPPH(2,2-diphenyl-1-picrylhydrazyl)(Sigma-Aldrich, Germany), Folin-Ciocalteu (Germany, applichem), Ethylene diamine tetra acetic acid (EDTA)(Sigma-Aldrich, Germany), Sodium Dihydrogen Phosphate (Germany, applichem) n-hexane (Germany, Merck) Di-Methyl sulfoxide (Germany, Merck), Sodium carbonate (Germany, appliChem),

Collection and Processing of Plant Material

Fresh leaves of *Sesuvium sesuvioides* plant were collected from the Mojgarh, Dingarh and Darawar fort areas of Cholistan, Bahawalpur region, Pakistan. *Sesuvium sesuvioides* was identified and confirmed by Department of Botany, Government College University Faisalabad. Leaves of *Sesuvium sesuvioides* were dried at normal room temperature for about 10 days. Then these air-dried leaves of plant were chopped down in smaller pieces. The leaves are crushed into small pieces to form powder. The powdered crude drug is stored in container for further use.

Extract preparation

300 g of air-dried powder were soaked in 1000 mL of ethanol, n-hexane and distilled water in three different jars. Jars were then sealed by aluminum foil and kept for 72 hours, administered to random stirring and shaking. Filtration of mixture was carried out by using Whattman No. 8 filter paper. The filtrates of different extracts were evaporated in rotary evaporator. The percentage yield of ethanolic extract, n-hexane and water extract was 3.9g, 2.7g and 3.5g respectively.

Determination of total phenolic contents (TPC)

Preparation of Standard Gallic Acid for Calibration Curve

The total phenolic contents in the ethanolic extracts of plants were described by Folin-Ciocalteu method as determined by (Shabbir, Khan, & Saeed, 2013). Different amounts of gallic acid were chosen in order to produce calibration curve. 1mL fractions of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.10mg/mL gallic acid solution in the ethanol were combined with the 5mL of Folin-Ciocalteu reagent (diluted ten folds) and the 4mL of Na2CO3 sodium carbonate (20%). The absorbance was determined after 1 hour at frequency of 765nm and curve of calibration was drawn by picking absorbance of substance as a function of concentration. 1mL of plants extract (0.001g/mL) was incorporated with similar reagent as determined above and then after 1-hour absorbance of blue color complex as a result was calculated at 765nm. All calculations were carried out in triplicate. Quantification was carried out by comparing values with the standard (gallic acid). Total amount of phenolic compounds in the extract of plant in gallic acid equivalents (GAE) were calculated by the following formula.

Preparation of Samples for Total Phenolic Content

Different extracts of Sesuvium sesuviodes of 75 μg/mL were prepared. The procedure as described for standard gallic acid was followed, and absorbance for each concentration of the extracts was recorded. The samples were prepared in triplicate for each analysis, and the average value of absorbance was used to plot the calibration curve to determine the level of phenolics in the extracts.

Total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). The total phenolic contents in all the samples were calculated by the using the formula:

Formula:

T = C x V / M

Where,

M = the weight of plants extract in grams

V = the volume of extract in mL.

T = total contents of phenolic compound in mg GAE/g plant extract.

C = the concentration of gallic acid obtained from calibration curve in mg/mL.

Total flavonoids contents

Preparation of Standard Quercetin for Calibration Curve

The total flavonoid amounts found in extract of plant were calculated according to the methodology determined by (Shabbir et al., 2013). Shortly 0.5mL of extract was combined with 0.15mL of 5% $NaNO_2$ solution and 2mL of purified water and settled down for 6 minutes.

After this, 0.15mL of 10% Aluminum chloride($AlCl_3$) solution was mixed to that and once again incubated for time duration of 6 minutes followed by incorporation of 4% sodium hydroxide(NaOH) solution to that mixture. Mass of mixture of reaction was prepared up to 5mL by incorporation of methanol in that and then shake well.

Absorbance of mixture of reaction was calculated at 510nm after settling for duration of 15 minutes. Total flavonoid contents (TFC) of the extract were expressed in terms of µg catechin equivalents per mL of extract of plant from linear regression curve of catechin.

Preparation of Samples for Total Flavonoid Content

Stock solutions of 4 mg/mL concentration in ethanol of the extracts were prepared, and they were diluted serially to make concentrations 0.75 mg/mL solutions. Similar procedure as described for quercetin was followed for the extracts also, and the absorbance was measured by spectrophotometer at 510 nm. Readings were taken in triplicate, and the average value of absorbance was used to calculate the total flavonoid content. The flavonoid content was expressed as quercetin equivalent (mg QE/g) using the linear equation based on the standard calibration curve.

Antioxidant Activities

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

Antioxidant Potential of *Sesuvium sesuvioides* extract was elaborated by the virtue of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as determined by (Dewan et al., 2013). In this method, 1mL of 0.004% DPPH in methanol solution (freshly prepared) was poured in the 3mL of plants extract and after that mixture solution was placed in dark for 30 minutes.

After that, absorbance was determined at frequency of 517 nm. Greater radical scavenging activity is indicated by low level of absorption by the reaction mixture. Ascorbic acid and BHT was taken as standard in order to analyze and compare antioxidant activity of drug of choice. For control chamber, solution without the plant extract was utilized (Zubair, Anwar, & Shahid, 2012). In order to achieve accuracy, all tests were carried out for 3 times. The inhibition of DPPH radical sample was determined in percentages by following equation.

Antibacterial Activity

Bacterial samples

The sample was individually examined against different bacterial strains, including **Gram-negative** bacteria: *Escherichia coli* (*E. coli*) ATCC 25922 and **Gram-positive** bacteria: *Bacillus subtilus* JS-2004 (*B. subtilis*) JS-2004, hygienity and recognition were confirmed by Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan.

Bacterial suspension Preparation

Bacterial strains were grown for a night at 37 ºC in plate carrying Nutrient agar (Oxoid, UK). The antibacterial mechanism of compounds found in extract was elaborated or examined by virtue of well diffusion method.

Shortly, one hundred µL of slurry of approved microorganisms, consisting of 10^7 CFU/mL of bacterial cells on medium of nutrient agar. Sample is added in the agar plates which had been incorporated with tested bacterial strains earlier.

Ciprofloxacin which is used as positive standard or reference for bacteria to correlate strain/isolate in assumed microbial strains. Plates, after placing two hours at four ºC, were incubated at temperature of thirty-seven ºC for eighteen hours for bacteria strains.
Antibacterial activity was examined by calculating diameter of growth inhibition zones (zone reader) in (mm) millimeters for microorganisms and then comparing these values to the standard or control.

Hemolytic activity:
Hemolytic activity of the plant extract was investigated by the methodology utilized by (Shabbir et al., 2013). Three mL freshly acquired heparinized bovine blood was assembled from Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan.

Blood was then preceded towards centrifugation process, centrifuged for almost five min at one thousand x*g*. Plasma was disposed and remaining cells were cleaned three times by using five mL of frosty (four degree centigrade) disinfected isotonic Phosphate-buffered saline (PBS) having pH 7.4. Erythrocytes were preserved 10^8 cells per mL for every assay. Hundred µL of each substance was incorporated with human (10^8cells/mL) individually. Samples were then incubated for thirty-five minutes at 37ºC and shaked after ten minutes. Rapidly after process of incubation the samples were then settled on ice for about five minutes then it undergo process of centrifugation that is centrifuged for about five minutes at 1000x*g*.

Supernatant 100 µL were separated from each and every tube and then it is diluted ten times with chilled PBS (4ºC). Triton X-100 (0.1% v/v) was chosen as positive control and on the other hand phosphate buffer saline (PBS) was chosen as negative control and undergo the same process. The absorbance was calculated at 576 nm using µQuant (Bioteck, USA). The % RBCs breakdown for each and every sample was determined.

Formula for hemolysis calculation:
% Hemolysis = Absorbance of sample – Absorbance of negative controls/ Absorbance of positive control × 100

Statistical analysis
Statistical analyses were done by using GraphPad Prism 8.1.0 (Graph Pad Software, Inc., USA). Linear regression analysis was used to calculate the IC_{50} values. $P < 0.05$ was taken as a level of statistical significance in all tests. The results were expressed as mean ± SEM (Standard Error of Mean).

Results and discussion
Total Phenolic contents
The total phenolic contents in the ethanolic extracts of plants were described by Folin-Ciocalteu method as determined by (Shabbir et al., 2013). Different amounts of gallic acid were chosen in order to produce calibration curve. The absorbance was determined after 1 hour at frequency of 765nm and curve of calibration was drawn by picking absorbance of substance as a function of concentration. Maximum effect of TPC (mgGAE/g) was shown by n-hexane extract of *Sesuvium sesuvioides* 172.4±1.88mg GAE/g. Ethanolic extract represented 168.6±1.33mg GAE/g activity and distilled water extract showed 167.3±.555mg GAE/g.

Table 1: Total phenolic contents (TPC) in different extracts of *Sesuvium sesuvioides*

Sr. No.	Sample	Mean TPC value (GAE/g)
1	SS-E	168.6±1.33mg GAE/g
2	SS-Nh	172.4±1.88mg GAE/g
3	SS-DW	167.3±.555mg GAE/g

Total Flavonoids contents

The total flavonoid amounts found in extract of plant were calculated according to the methodology determined by (Shabbir et al., 2013). Absorbance of mixture of reaction was calculated at 510nm after settling for duration of 15 minutes. Total flavonoid contents (TFC) of the extract were expressed in terms of µg Catechin equivalents per mL of extract of plant from linear regression curve of Catechin (Y= 0.0011x; R^2 =0.992). n-hexane extract showed maximum value of TFC 21.428±1.33 mg QE/g), followed by ethanolic extract 20.658±1.44 mg QE/g and distilled water extract 19.427±2.11 mg QE/g.

The results of total flavonoid content (TFC) assay reported the highest presence of flavonols in SS.

Table 2: Total Flavonoids Contents (TFC) in different extracts of *Sesuvium sesuvioides*

Sr. No.	Sample	Mean TFC value (QE/g)
1	SS-E	20.658±1.44 mg QE/g
2	SS-Nh	21.428±1.33 mg QE/g)
3	SS-DW	19.427±2.11 mg QE/g

Antioxidant Activity of Extract by DPPH Scavenging Activity:

Antioxidant Potential of *Sesuvium sesuvioides* extract was elaborated by the virtue of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as determined by Shahid *et al*.(2014). DPPH is widely used to determine the anti-oxidant potential of natural compounds or drugs (Sujarwo and Keim 2019) and showed radical scavenging ability In current research work, Ss showed signifcant scavenging capability by DPPH and methods. The antioxidant capacity of this plant indicated the presence of phenolic compounds with a number of hydroxyl groups (Lekouaghet et al. 2020)

In this method, 1mL of 0.004% DPPH in methanol solution (freshly prepared) was poured in the 3mL of plants extract and after that mixture solution was placed in dark for 30 minutes. After that, absorbance was determined at frequency of 517 nm. Greater radical scavenging activity is indicated by low level of absorption by the reaction mixture.

Ascorbic acid and BHT was taken as standard in order to analyze and compare antioxidant activity of drug of choice. Maximum % inhibition of the ethanol extract was 66.667 %, whereas IC_{50} ± SEM (µg/mL) was 81.48±1.22. Results showed that SS E had a significant free radical reduction capability then SS nh represented anti-oxidant activity (64.047) whereas IC_{50} ± SEM (µg/mL) was79.38±1.55, and then SS DW 63.532% whereas IC_{50} ± SEM (µg/mL) was 73.48±1.433.

The phytochemical studies revealed the presence of flavonoids, phenols, coumarin, glycosides, terpenoids, fats, saponins and carbohydrates in plant (Sajid-Ur-Rehman et al., 2021). Flavonoids can prevent tissue injury that cause by free radicals. They have free radical scavenging activities. Radical oxidised the flavonoids resulting in the formation of more less stable reactive radical. Hanasaki *et al*. (Hanasaki, Ogawa, & Fukui, 1994) found that some of the flavonoids can directly scavenge superoxides, whereas other flavonoids can scavenge the highly reactive oxygen-derived radical called peroxynitrite.

Table 3: DPPH Scavenging Activity of Medicinal Plant Extract

Sr. No.	Extraction| Fraction	% Inhibition of DPPH	IC_{50} ± SEM (µg/mL)
1	SS E	66.667	81.48±1.22
2	SS nh	64.047	79.38±1.55
3	SS DW	63.532	73.48±1.433

Mean ±SD absorbance values of DPPH

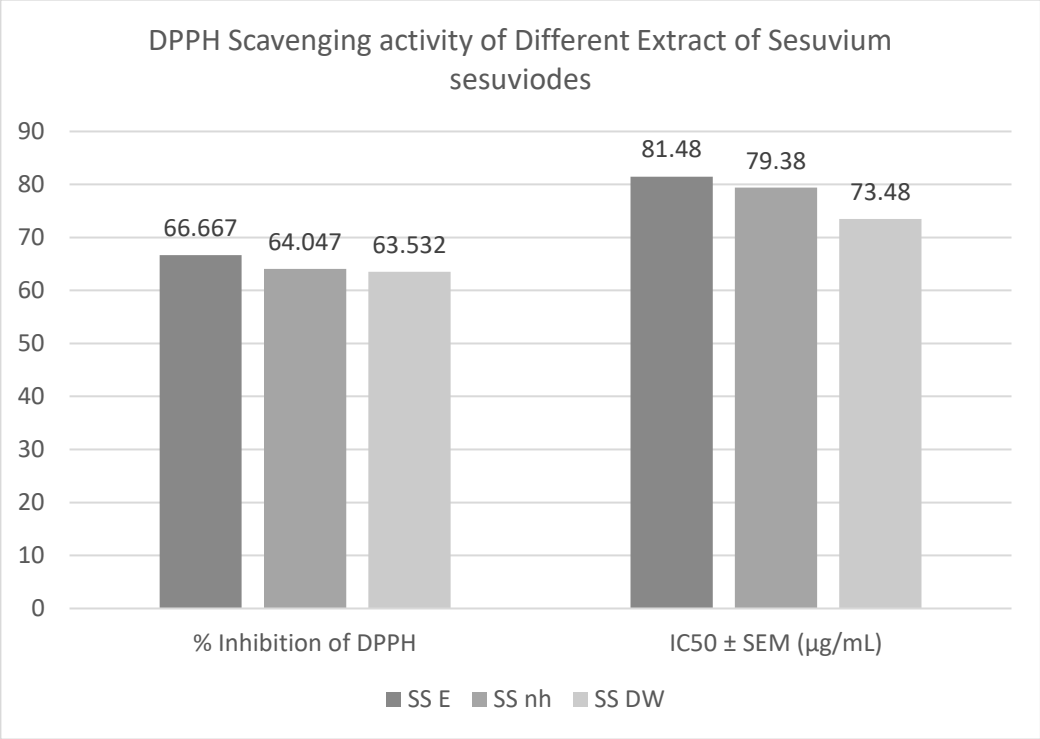


Figure 1: Antioxidant Activity by DPPH

Antibacterial Activity of different extract of *Sesuvium sesuvioides*

The sample was individually examined against different bacterial strains, including Gram-negative bacteria: *Escherichia coli* (*E. coli*) and Gram-positive bacteria: *Bacillus subtilus* (*B. subtilis*). In general, the standard drug Ciprofloxacin produced [(24 ±0.33) mm] zone of inhibition. Among three extracts of *Sesuvium sesuvioides*, major zone of inhibition was shown by distilled water extract of *Sesuvium sesuvioides* [(18±0.333) mm], then n-hexane extract showed [(13.5±1.52)] mm zone of inhibition and after that ethanolic extract exhibit [(11±0.33) mm] of zone of inhibition. In same way, distilled water extract of *Sesuvium sesuvioides* showed greater inhibitory zone [(19±1.0) mm] than other extracts. The n-hexane extract gave [(14±1.0) mm] inhibitory zone against B. subtilis.

And ethanolic extract represented least activity as compared to others extracts [(8±0.33) mm]Flavonoids are polyphenolic compounds exhibited antibacterial potential via different mechanisms of action. According to different research studies, flavonoids can suppress nucleic acid synthesis, cytoplasmic membrane function and energy metabolism (Xie, Yang, Tang, Chen, & Ren, 2015). Flavonoids also been able to reduce adhesion and formation of biofilm, permeability of membrane, porin on cell membrane and pathogenicity, all of these are crucial for the growth of bacterial (Biharee, Sharma, Kumar, & Jaitak, 2020). In addition, some flavonoids have been reported to reverse antibiotic resistance and improve the efficacy of the present antibiotics (Biharee et al., 2020).

Table 4: Anti-bacterial activity of *Sesuvium sesuvioides* against *E. coli* & *B. subtilis*

Sr. No.	Plant Extracts	*E. coli* (Gram Negative) Zone of Inhibition (mm)	*B. subtilis* (Gram Positive) Zone of Inhibition(mm)
1	SS-E	11±0.33	8±0.33
2	SS-Nh	13±1.52*	14±1.0*
3	SS-DW	18±0.333**	19±1.0**

Data are shown as mean±SD (**P<0.001, *P<0.01)

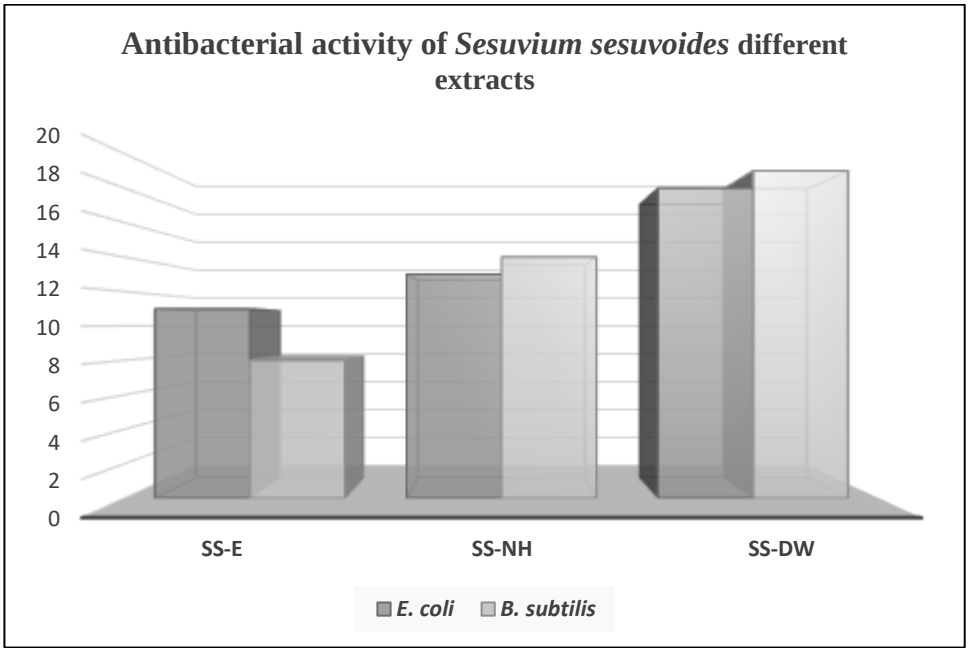
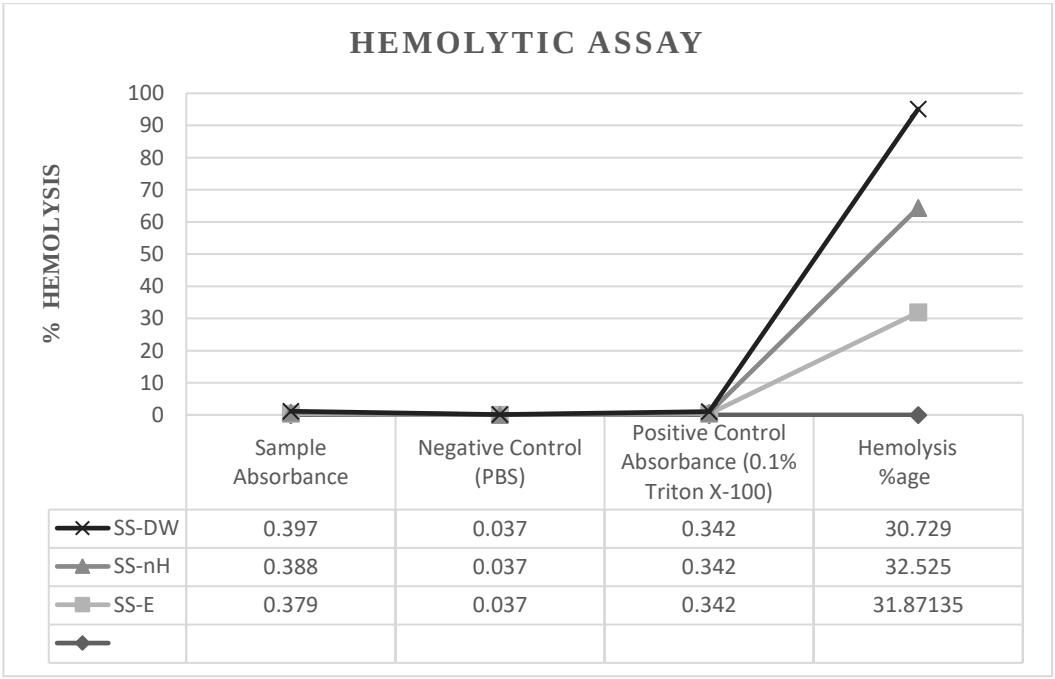


Figure:2

Hemolytic assay

Erythrocytes cells are present in large amount in human body with their own morphological, biological and replicative characteristics. The hemoglobin and polyunsaturated fatty acids mainly target the erythrocytes due to their redox-active oxygen transportation feature. As a result, hemolysis of erythrocyte membrane occurs by oxidation.

Hemolytic activity of *S. sesuviodes* extracts was evaluated against bovine erythrocytes to assess the effect of different extracts on erythrocyte membrane integrity. All extracts exhibited low hemolytic activity as activity in all extracts was less than 10%. In case of cytotoxic effect, n-hexane extract showed more hemolytic activity 32.525%, While ethanol and distilled water extract showed very low hemolytic activity i.e. 31.871% and 30.729% respectively.



	Sample Absorbance	Negative Control (PBS)	Positive Control Absorbance (0.1% Triton X-100)	Hemolysis %age
SS-DW	0.397	0.037	0.342	30.729
SS-nH	0.388	0.037	0.342	32.525
SS-E	0.379	0.037	0.342	31.87135

Figure 3: Hemolytic activity of *Sesuvium sesuvioides*

Conclusion

The present study demonstrates the *in vitro* anti-oxidant, antimicrobial and hemolytic activity of different extract of *Sesuvium sesuviodes.* Extracts with higher antioxidant capacity also had higher polyphenol content. The different extracts of *Sesuvium sesuviodes* exerts stabilizing effects on reactive oxygen species (ROS), inhibiting gram positive gram negative bacterial strains and promoting cytotoxicity. Maximum effect of TPC (mgGAE/g) and TFC was shown by n-hexane extract of *Sesuvium sesuvioides.* n-hexane extract showed more hemolytic activity then other extracts. Results showed that SS E had a significant free antioxidant (radical reduction capability). The result of the study showed that unexplored *Sesuvium sesuviodes* exhibited significant antimicrobial, antioxidant and cytotoxic potential. However the evaluation and the discovery of new agents is a process of long term that consists many steps by step approaches with the detailed screening of plant, followed by the isolation, characterization and identification of bioactive compounds. Therefore further research is required to explore the plants and its phytoconstituents.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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